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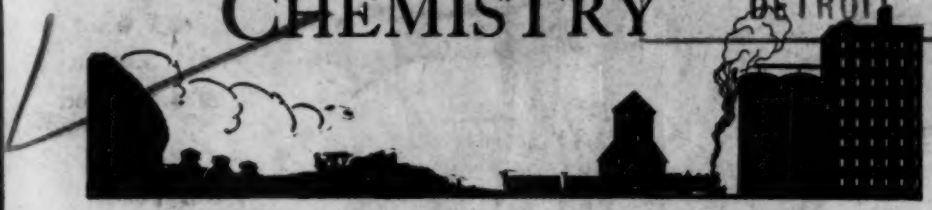

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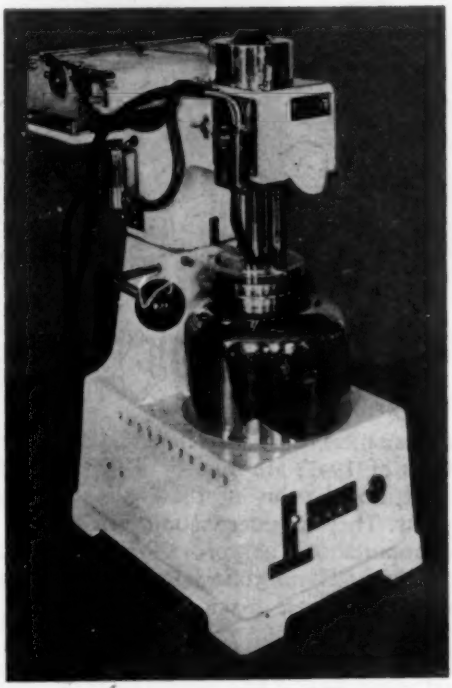

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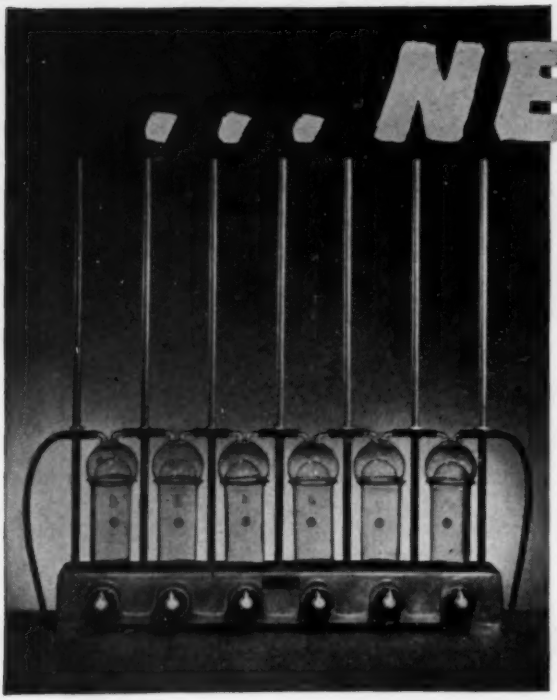

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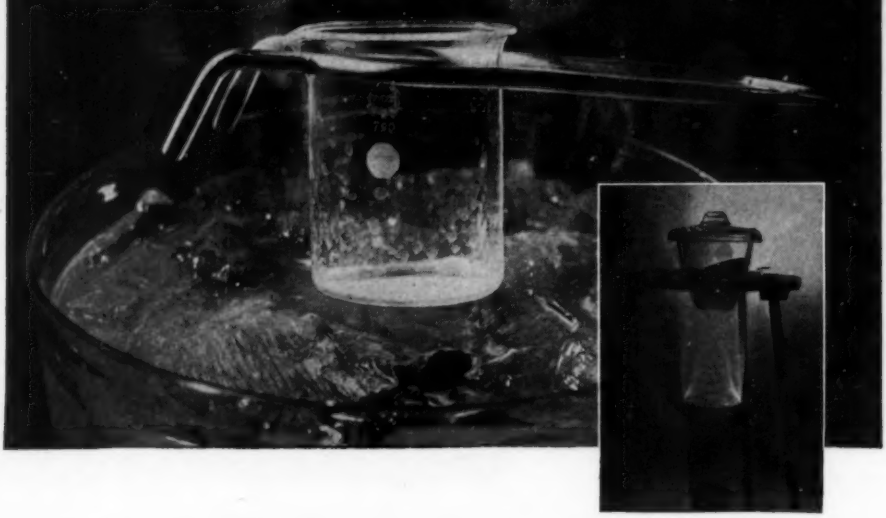

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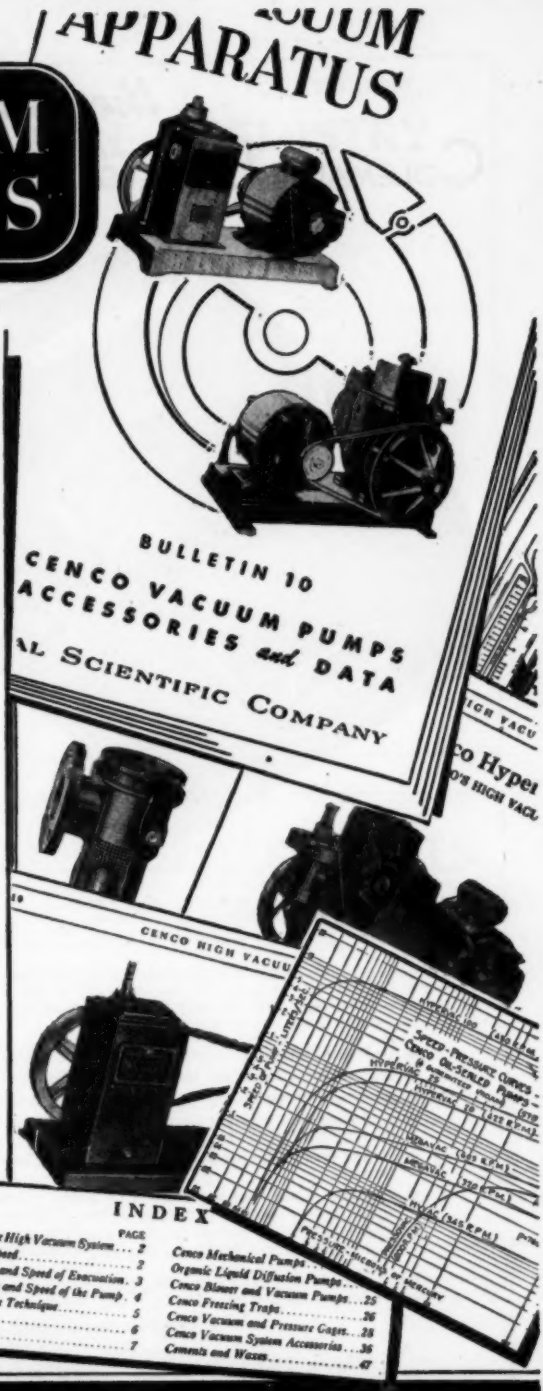

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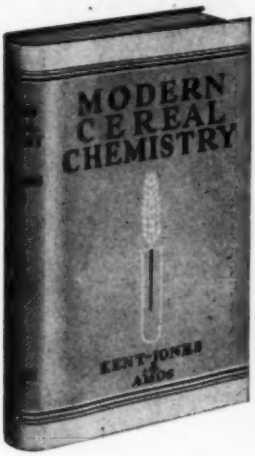

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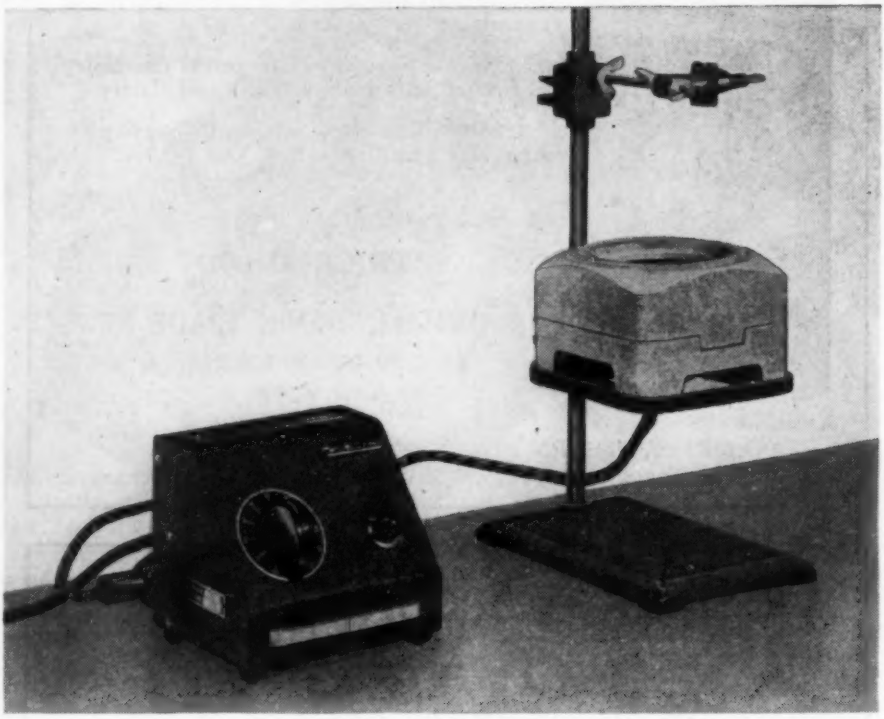

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CEREAL CHEMISTRY

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STUDIES ON BREAD STALING. I. THE ROLE OF STARCH [1]

THOMAS JOHN SCHOCH and DEXTER FRENCH [2]

Corn Products Refining Company, Argo, Illinois

(Presented at the annual meeting, May 1946; received for publication February 24, 1947)

The staling of bread has been of major interest to the starch chemist, partly because starch constitutes the principal constituent of bread and partly because reactions occur in simple starch systems which bear at least a superficial resemblance to staling. During the latter process, the starch substance of bread becomes less soluble, seemingly by spontaneous transformation from a sol state to a crystalline aggregate. It appears that Lindet (1902) originated the term "retrogradation" to describe this change in the starch substance of bread. The same term was later employed by Maquenne and Roux (1905) to designate various insolubilization phenomena of starch itself. These include the gradual increase in viscosity and opacity of starch pastes and solutions, the spontaneous precipitation of starch from solution, and the irreversible gelation of aqueous starch systems. Such effects are explicable on the basis of aggregation of high-polymeric starch molecules through associative forces, to give a particle too large to remain in solution. In support of this theory, Katz (1930, 1934) has shown that staled bread and retrograded starch both possess B-type X-ray patterns, suggestive of side-by-side association of linear starch molecules.

Recent concepts (Meyer, 1942) picture the normal starches as composed of two polymeric carbohydrates, a linear chain of some 400–1000 glucose units (the A-fraction or amylose) and a branched or treelike molecule of perhaps several thousand glucose units (the B-fraction or amylopectin). The first component exhibits the various retrogradation effects of starch to a greatly exaggerated degree; the second component is colloidally stable and shows little evidence of aggregation or insolubilization. Consequently, it has been assumed

[1] This work was conducted as a project under the sponsorship of the Committee on Food Research, Office of the Quartermaster General.

[2] Present address: Department of Chemistry, Iowa State College, Ames, Iowa.

that the linear component is responsible both for the retrogradation of starch pastes and for the staling of bread. For example, Hixon (1943) speculates that if a waxy variety of wheat were known, bread baked from such a flour might not be susceptible to staling, since the waxy starches contain no linear-chain component.

On the other hand, Alsberg (1927, 1928) has questioned whether staling can be attributed solely to retrogradation, pointing out that staling is a rapid process, while retrogradation is much slower. Also, staled bread is restored to its original freshness merely by heating above 50°C, and the cycle of staling and refreshening can be repeated indefinitely. This behavior is not in accord with the irreversibility usually ascribed to retrogradation of the linear component. MacMasters *et al.* (1946) have observed that pastes of waxy maize starch (containing only the branched B-fraction) become insoluble when frozen and thawed, so that the resulting starch "sponge" does not redissolve at room temperature. Noznick, Merritt, and Geddes (1946) have prepared a synthetic flour from waxy maize starch and nondevitalized gluten; bread baked from this flour showed normal tendency to stale. From these observations it would seem that the linear A-fraction is perhaps not essential to the staling process and that the branched B-fraction by itself may undergo a sort of spontaneous insolubilization.

In view of the major advances in starch chemistry since the studies of Katz, particularly the isolation and characterization of the pure fractions, it seemed advisable to reexamine some of these concepts of bread staling. The purposes of present studies were threefold: (1) to determine which of the starch fractions is responsible for staling, (2) to clarify the mechanisms involved, and (3) to evaluate the influence of other bread constituents (proteins, shortening, salt) on the staling process.

Procedures

Nature of the Soluble Starch from Bread. It is well known that an appreciable amount of the starch in fresh bread is in a soluble state and can be extracted from the crumb with cold water. During staling, the amount of this soluble starch decreases progressively; in fact, this effect has been used as a criterion for estimating the extent of staling (Katz, 1928). At the commencement of present studies, it was felt that a more critical examination of this soluble starch should provide additional information on the changes that occur in the starch substance of bread during the staling process. Consequently, the solubles have been extracted from bread at various stages of staling by leaching with water at 30°C. The amount of this soluble carbohydrate and its

iodine affinity have been determined in order to trace the fate of the individual starch fractions during staling. Incidental to these studies, a modified procedure has been developed for evaluating the swelling power of bread crumb.

Wrapped sliced sandwich bread was obtained locally within a few hours after baking and stored at 21°C and 60% relative humidity. Due to development of mold, tests could not be carried beyond 6 days. After various periods of storage, 25.0-g samples (on as-is basis) were taken for analysis, using the center of the loaf and discarding one-half inch of crust and outer crumb. Simultaneously, a sample was taken for moisture determination, drying for 4 hours in the vacuum oven at 70°C. The 25-g sample was broken up and placed in a tared 250-ml Pyrex centrifuge bottle. Approximately 150 ml of distilled water was added and the mixture stirred vigorously for one minute with a propeller-type stirrer to give a smooth paste.[3] The bottle was placed in a thermostated bath at 30°C for 30 minutes, the contents being gently stirred during this period to keep the bread crumb suspended. The mixture was then centrifuged for 5 minutes at 2000 RPM, the supernate decanted, and the bottle plus sediment weighed. The sediment was resuspended in 100 ml of distilled water and again digested for 30 minutes in the 30°C bath. After centrifuging, the supernate was decanted and the bottle and sediment again weighed. Sediment weights were averaged[4] and the swelling power calculated as the weight of sediment per gram (dry basis) of bread. The sediment was extracted a third time in similar fashion, and the three decantates combined and filtered to remove insolubles and fatty material. The filtrate and wash waters were treated with 3–4 volumes of methanol to flocculate the soluble starch, the mixture heated on the steam bath for an hour, then allowed to settle overnight. The precipitate was collected in a weighed Gooch crucible, washed with alcohol, and dried in the vacuum oven. The percentage of solubles was calculated on the dry-basis bread. To remove traces of fatty material which might interfere with estimation of iodine affinity, the soluble starch was Soxhlet-extracted for 16 hours with methanol. Iodine affinities were determined by a modified potentiometric method.[5] Duplicate extractions were made in each case, and results are summarized in Table I.

[3] An attempt was made to use the Waring Blendor to break up the crumb structure, but this treatment produced extensive rupture of the starch granules. After two minutes of "blending," microscopic examination showed that most of the granules had been disintegrated. Likewise, the action of the "Blendor" caused a progressive increase in the swelling power of the crumb.

[4] In most cases, the agreement between the first and second weighings of the sediment was within experimental error. Precision of measurement of swelling power is of the order of 1% (i.e., average deviation from the mean is 11 parts per 1000).

[5] Method of Bates, French, and Rundle (1943), modified by Wilson, Schoch, and Hudson (1943).

TABLE I

EFFECT OF STALING ON THE SWELLING POWER OF BREAD CRUMB AND THE QUANTITY AND COMPOSITION OF SOLUBLES EXTRACTABLE AT 30°C

Age	Moisture	Swelling power	Solubles	I_2 affinity of solubles	Estimated composition of solubles[1]	
					A-fraction[2]	B-fraction[3]
Hours	%		%		%	%
4	42.6	3.61	6.21	1.91	0.60	5.61
			7.94	2.17	0.87	7.07
6	43.6	3.67	7.12	2.04	0.73	6.39
			7.40	2.31	0.86	6.54
28	41.0	3.06	4.74	1.66	0.40	4.34
			4.85	1.63	0.40	4.45
76	39.8	2.72	3.48	1.45	0.25	3.23
			3.65	1.45	0.27	3.38
148	37.3	2.64	3.06	1.53	0.24	2.82
			3.10	1.33	0.21	2.89

[1] Calculated to dry bread basis.

[2] Calculated on basis of 19.9% iodine affinity for pure wheat A-fraction, viz., % A-fraction $= \frac{\%\ \text{solubles} \times \text{iodine affinity of solubles}}{19.9}$.

[3] By difference.

It was of interest to ascertain how the refreshening of staled bread by moderate heating might influence the yield and character of the soluble starch. It would be difficult to standardize conditions of refreshening by merely heating the staled bread in an oven, owing to different moisture content of the various samples. Hence refreshening was effected by conducting the extraction of soluble starch at 50°C, using the same procedure as for 30°C. Data on swelling power at 50°C and amount and iodine affinity of the solubles are presented in Table II.

Fractionation of Wheat Starch. Since iodine affinity was employed to establish the composition of the soluble extract at various stages of staling, it was necessary to determine the iodine affinity of defatted wheat starch and of pure wheat A-fraction. The wheat starch employed for this purpose was from an experimental batch prepared at the Northern Regional Research Laboratory by SO_2 steeping. Preliminarily, this starch was defatted by four successive digestions with boiling 85% methanol, each such digestion for a period of 3 hours. Its iodine affinity by the modified method of Whistler and Hilbert (1945) was 5.21 ± 0.07%. This defatted starch was fractionated by gelatinizing in hot water to give a 2% paste; the latter was autoclaved for 2 hours at pH 6.5, 10% by volume of "Pentasol" added (Sharples

brand mixed amyl alcohols), and the mixture allowed to cool to room temperature with continuous stirring. The crude A-fraction was collected in the Sharples supercentrifuge, dissolved in hot water in the presence of excess n-butyl alcohol, and the hot solution supercentrifuged to remove extraneous impurities. The recrystallized A-fraction separated on cooling; it was repeatedly recrystallized by solution in hot water and precipitation with butyl alcohol, until the iodine affinity reached a maximum value of 19.9%. Experimental yields of A-fraction were not determined with sufficient care. However, the content of linear component can be calculated as 26% from the iodine affinities of the defatted starch and the pure A-fraction (viz., 5.21% divided by 19.9%). Two batches of wheat B-fraction had iodine affinities of 0.56% and 0.44%.

Nature of the Soluble Starch from 50% Pastes of Defatted Wheat Starch. Bread represents a highly complex system, in which both physical and chemical interactions may occur between the various components. To avoid such complications, it seemed logical to transpose these studies to simple starch-water pastes. If the swelling power and the amount and iodine affinity of the solubles show the same trends during the aging of simple starch pastes as during the staling of bread, then these effects must be inherent in the starch itself and not due to the influence of other bread constituents.

Consequently, subsequent aging studies were conducted on defatted wheat starch pastes, prepared under conditions to simulate the

TABLE II

EFFECT OF STALING ON THE SWELLING POWER OF BREAD CRUMB AND THE QUANTITY AND COMPOSITION OF SOLUBLES EXTRACTABLE AT 50°C

Age	Moisture	Swelling power	Solubles	I_2 affinity of solubles	Estimated composition of solubles	
					A-fraction	B-fraction
Hours	%		%		%	%
4	42.6	3.75	9.04	3.06	1.39	7.65
			9.64	2.95	1.43	8.21
6	43.6	4.02	7.41	2.73	1.02	6.39
			7.70	2.78	1.08	6.62
28	41.0	3.78	8.04	2.72	1.10	6.94
			8.04	2.80	1.13	6.91
76	39.8	3.68	7.48	2.90	1.09	6.39
			7.89	2.64	1.05	6.84
148	37.3	3.73	7.70	2.56	0.99	6.71
			8.09	2.74	1.11	6.98

baking and cooling of the loaf of bread. The concentration of the paste was arbitrarily fixed at 50% (dry starch basis). Confirming Alsberg's statement (1927, 1928) that all the starch granules of bread are completely gelatinized, observations of moistened bread crumb under the polarizing microscope showed uniform swelling and no birefringent granules. Some difficulty was encountered in effecting similar uniformity in the gelatinization of 50% wheat starch suspensions. When a test tube containing such a suspension was rapidly heated in a boiling water bath, the starch adjacent to the walls of the tube gelatinized immediately, withdrawing water from the interior of the suspension and leaving a core of ungelatinized starch in the center of the tube. However, when the starch suspension was slowly heated, uniform gelatinization of the granules occurred throughout the suspension. To duplicate the temperature rise during the baking of bread, a standard loaf was baked with a thermometer imbedded in the center of the dough. In close agreement with Barackman and Bell (1938), the temperature rose to 100°C over a period of about 20 minutes, then remained at this level for the remaining 20–30 minutes of baking. Similar conditions were therefore used for the pasting of 50% starch suspensions.

An amount of the defatted wheat starch equivalent to 10.0 g on dry starch basis was weighed into a 25 × 100 mm test tube and sufficient water added to give a total weight of 20.0 g (i.e., 10 ml of water less the moisture of the starch). The mixture was thoroughly stirred with a small nickel spatula until homogeneous, the spatula removed, and any adhering starch scraped back into the tube. The latter was stoppered with a vented rubber cap and placed in a water bath, the temperature of which was raised at a uniform rate to 100°C over a period of 30 minutes. The paste was heated in the boiling water bath for an additional 30 minutes, then allowed to cool slowly to room temperature. Under these conditions, microscopic examination showed only a few ungelatinized and birefringent granules, probably representing less than 2% of the total starch.

The resulting gels were hard, elastic, and almost cartilaginous in character. Extraction studies were conducted on these pastes after cooling to room temperature (i.e., 0 days) and after 3 and 6 days' storage at 21°C. To prepare the sample for extraction, the gel (representing 10.0 g of starch) was removed from the tube and pressed through a 40-mesh screen, then rinsed quantitatively into a tared 250-ml Pyrex centrifuge bottle with 150 ml of water. Swelling power, amount of soluble starch, and iodine affinity of the solubles were determined on starch pastes aged for 0, 3, and 6 days, the extractions in each case being conducted at 30°C and at 50°C (Table III). The

technique of extraction was similar to that described above for bread samples, except that the combined decantates were precipitated directly without filtering, and Soxhlet extraction of the soluble starch was omitted.

Effect of Adjuncts on the Aging of Starch Pastes. This technique of operating on simple starch pastes permits evaluation of the influence of other bread constituents on the staling process. Similar tests have therefore been made on the aging of 50% defatted wheat starch pastes,

TABLE III

EFFECT OF VARIOUS BREAD INGREDIENTS ON THE STALING OF 50% WHEAT STARCH PASTES, AS MEASURED BY CRUMB SWELLING POWER AND PERCENTAGE OF SOLUBLES

Adjunct	Age	Extraction at 30°C			Extraction at 50°C		
		Swell	Solubles	I_2 affinity	Swell	Solubles	I_2 affinity
	Days		%			%	
Defatted wheat starch	0	5.05 5.17	3.03 2.85	3.4 3.5	5.37 5.48	3.15 3.51	4.5 4.5
No adjunct	3	3.63 3.61	1.02 1.00	3.4 2.9	5.16 5.02	3.15 3.06	3.6 4.0
	6	3.57 3.52	1.05 1.04	3.3 3.5	4.95 5.40	2.66 2.74	3.7 4.0
Defatted wheat starch+2% salt	0	4.55 4.51	2.66 2.54	3.7 3.7	5.31 5.22	3.98 4.68	4.1 4.3
	3	3.53 3.54	1.11 1.09	2.9 3.3	5.10 4.87	3.00 3.15	4.1 4.2
	6	3.53 3.55	0.97 0.87	3.1 3.0	4.72 4.95	2.43 2.61	4.1 4.1
Defatted wheat starch+2% yeast	0	4.88 4.71	2.99 3.10	3.6 3.3	5.38 5.37	4.68 4.47	3.8 3.8
Defatted wheat starch+25% egg albumen	0	5.42 6.33	3.64 4.12	4.2 4.5	6.57 6.22	4.14 6.36	5.5 5.1
	3	5.07 4.90	1.55 1.75	3.5 3.6	6.02 6.01	3.33 3.06	4.5 5.1
	6	4.74 4.84	1.19 1.26	3.8 4.1	5.88 5.90	2.89 2.75	4.5 4.5
Defatted wheat starch+10% casein[1]	0	4.58 4.48	2.57 2.44	4.0 2.0	4.99 5.12	3.38 3.75	5.8 5.0
	3	3.44 3.48	0.88 1.00	3.9 4.0	4.77 4.85	2.91 3.20	5.2 4.8
	6	3.48 3.62	1.45 2.26	1.2 1.8	4.55 4.52	3.69 3.47	3.5 4.0

TABLE III—*Continued*

Adjunct	Age	Extraction at 30°C			Extraction at 50°C		
		Swell	Solubles	I_2 affinity	Swell	Solubles	I_2 affinity
	Days		%			%	
Defatted wheat starch+4% hydrogenated shortening	0	4.32	2.59	3.3	4.81	3.67	4.9
		4.33	2.57	4.1	4.96	3.42	4.0
	3	3.67	0.91	3.7	4.54	2.53	4.3
		3.55	0.97	3.2	4.51	2.43	4.9
	6	3.54	0.79	2.9	4.58	2.37	5.1
		3.48	0.76	2.6	4.55	2.61	3.8
Defatted wheat starch+4% corn oil	0	4.91	2.94	3.2	5.51	3.60	4.7
		4.79	3.07	2.9	5.60	3.25	4.4
	3	3.70	1.23	2.1	4.93	2.79	3.0
		3.62	1.14	1.9	4.87	2.73	3.0
	6	3.54	0.89	0.7	4.64	2.49	2.9
		3.57	0.90	0.9	4.66	2.53	2.8
Defatted wheat starch+2% oleic acid	0	3.83	1.29	0.5	4.09	2.25	<0.2
		3.81	1.54		4.05	1.68	
	3	—	—	—	3.94	1.83	[2]
		—	—	—	3.91	1.79	[2]
	6	3.22	1.17	[2]	3.88	1.99	[2]
		3.17	1.20	[2]	3.88	1.96	[2]
Defatted wheat starch+0.2% malt extract	0	3.49	11.09	1.6	4.12	11.44	1.8
		3.32	10.65	1.6	3.92	11.94	1.9
	3	3.14	6.95	1.4	3.89	12.58	1.8
		3.16	6.66	1.5	4.05	12.21	1.8
	6	3.22	4.11	1.7	4.16	9.84	1.8
		3.24	3.95	1.6	4.18	9.66	1.6

[1] Nitrogen was determined on each extract by a micro-Kjeldahl procedure. Values for % solubles represent total solubles minus the computed protein. Iodine affinities are corrected to starch basis.

[2] These samples were not analyzed for iodine affinity, since they gave a violet or red color with iodine, indicating an iodine affinity less than 0.6%.

but with the incorporation of various adjuncts to simulate the non-starchy constituents of bread. Details of processing are as follows:

SALT (2%): Pastes of defatted wheat starch were prepared as described above, except that 2% sodium chloride (on dry starch basis) was added to the suspension before pasting.

YEAST (2%): Dry yeast was incorporated in the mixture before pasting, to the extent of 0.2 g per 10 g of starch. Since the pastes did not show any substantial difference from defatted wheat starch, tests were not conducted beyond zero days.

EGG ALBUMEN (25%): Fifty g of "soluble egg albumen" was stirred into 200 ml of water, the mixture allowed to stand overnight in the refrigerator, then centrifuged to remove insolubles, and the pH adjusted to 6.5 with potassium hydroxide solution. Ten g (dry basis) of defatted wheat starch was mixed with 12.5 g of this albumen solution, and the mixture gelatinized, aged, and extracted as previously described. The egg albumen was completely insolubilized during the pasting operation and the aqueous extracts at both 30° and 50°C were substantially nitrogen-free.

CASEIN (10%): Ten g of acid-precipitated casein was dissolved in 100 ml of water and the pH adjusted to 6.3 with potassium hydroxide solution. Eleven g of this solution was mixed with 10.0 g (dry basis) of defatted wheat starch and the mixture pasted. Since the casein was not insolubilized by heat, it was extracted in the solubles and precipitated by alcohol. Kjeldahl analyses were therefore run on the extracted solubles and the starch calculated by difference. The accuracy of such a procedure is questionable, but there was no feasible method for effecting a quantitative separation between soluble starch and casein. Iodine affinities were determined on the total solubles and corrected to starch basis. Separate tests showed that the iodine affinity of wheat A-fraction is not appreciably affected by the presence of dissolved protein (i.e., casein or gelatin) in quantities as high as five times the amount of A-fraction being analyzed.

SHORTENING (4%): Six g of hydrogenated vegetable shortening was dissolved in 150 ml of hexane, thoroughly mixed with 150 g of defatted wheat starch, then the hexane removed on the steam plate with frequent stirring. Moisture content of the mixture was determined and pastes made up to contain 10.0 g starch, 0.4 g shortening, and 10 g water. Prior to evaluation of iodine affinity, the soluble starch was extracted overnight with ethyl alcohol.

CORN OIL (4%): Twelve g of refined corn oil and 300 g of defatted wheat starch were blended for one hour in a Hobart mixer. Subsequent procedure duplicated that employed with hydrogenated vegetable shortening.

OLEIC ACID (2%): This amount was sufficient to form an insoluble complex with all of the A-fraction of the wheat starch (Schoch and Williams, 1944). Three g of oleic acid was blended into 150 g of defatted wheat starch, and the pastes made up to contain 10.0 g starch, 0.2 g oleic acid, and 10 g water. The soluble starch was Soxhlet-extracted for 36 hours with ethyl alcohol. Due to low iodine affinity, it was necessary to combine the duplicate solubles from zero days aging, to provide sufficient material for iodine titration. The solubles from 3- and 6-day old pastes were tested qualitatively in each case

and found to give a red to red-violet color with iodine, indicative of negligible iodine affinity.

MALT EXTRACT (0.2%): 0.4 ml of diastatic malt extract (250° Lintner) was dissolved in 200 ml of water. 10.0 g of defatted wheat starch was then pasted with 10 ml of this solution. Judging from Lintner value, this enzyme level probably exceeds that of a diastated flour.

Discussion of Results

In order to interpret results on the extraction of bread, it is necessary to recall the classical fractionation of starch by selective leaching methods. When an aqueous starch suspension is heated to a temperature just sufficient to gelatinize the granules (as indicated by loss of birefringence) without excessive swelling, a portion of the A-fraction dissolves and diffuses into the aqueous substrate. If the swollen granules are then removed by sedimentation or centrifugation and the soluble carbohydrate precipitated from the supernate with alcohol, this material (termed the "amylose" by K. H. Meyer) will have an iodine affinity as high as 14%, indicating a preponderance of the linear-chain A-fraction. Correspondingly, the insoluble residue of swollen granules (Meyer's "amylopectin") will have an iodine affinity lower than that of the whole starch (Schoch, 1945).

It might be expected that a similar situation would occur during extraction of freshly baked bread, whereby the A-fraction would be leached from the swollen but intact granules. However, the iodine affinity of the solubles from fresh bread (Table I) is far below that of defatted wheat starch (i.e., 5.21%). The A-fraction must therefore be rendered nonavailable during baking and cooling of the loaf, probably by one or more of the following reactions:

1. The A-fraction may retrograde to insoluble state during baking of the loaf, thereafter resisting aqueous extraction.
2. The A-fraction may be immobilized as an insoluble complex with other bread constituents, such as fat or protein.
3. The A-fraction may be hydrolyzed by flour enzymes during the early stages of baking (e.g., after the temperature is sufficiently high to gelatinize the granules but insufficient to inactivate the enzyme).

This behavior is not peculiar to bread, since the solubles from freshly prepared 50% pastes of defatted wheat starch likewise have an iodine affinity lower than that of the parent starch (Table III). In the absence of fat, protein, and enzymes, this effect can only be attributed to retrogradation of the A-fraction during pasting and cooling of the

starch paste. Similarly, most of the A-fraction of fresh bread must retrograde during baking and cooling of the loaf. It seems unlikely that the very minor amount of A-fraction still in available soluble form could be responsible for subsequent staling. The soluble starch from fresh bread is greater in amount and lower in iodine affinity than the solubles from 50% starch pastes. At a later point, these differences will be attributed to the influence of flour enzymes and shortening.

The percentage of solubles extractable at 30°C decreases progressively with staling of the bread (Table I). There is a slight decrease in iodine affinity, indicating retrogradation of the small amount of A-fraction still in solution. However, this effect is considered negligible. The more important action is a progressive decrease in the amount of B-fraction leached from the bread, indicating a spontaneous insolubilization of this material during staling. Here, again, the same trend is duplicated with 50% pastes of defatted wheat starch (Table III); hence the effect is not due to the influence of other bread constituents. This behavior suggests (1) that the staling of bread is due to a progressive spontaneous aggregation of the branched-chain component and (2) that the linear fraction has no influence on staling, since it is already in an inert retrograded form.

This theory receives added confirmation from extraction studies at 50°C. While it was later realized that a somewhat higher temperature (e.g., 55°C) might have given more ideal results, the "refreshening" of both staled bread (Table II) and staled starch pastes (Table III) is obvious at 50°C. The swelling power and percentage of solubles are restored to high and fairly constant levels. The iodine affinity of the solubles, while higher than values obtained by extraction at 30°C, is still substantially lower than that of defatted wheat starch. Thus, solubilization of retrograded A-fraction cannot play any important part in the refreshening of staled bread.

If spontaneous insolubilization of the B-fraction is responsible for the decrease in swelling power and solubles on aging of starch pastes, then these effects should be observed with waxy maize starch. However, freshly prepared 50% pastes of defatted waxy maize starch were glutinous and slimy and could not be processed by the analytical procedures employed for bread and for 50% wheat starch pastes. On aging for 3–6 days, these pastes were transformed into hard tough gels, of much the same character as 6-day-old wheat starch pastes. When these staled gels were heated to 100°C, they reverted to slimy pastes. Hence the phenomena of staling and refreshening are duplicated at least qualitatively with waxy maize starch, containing no A-fraction.

While the solubles from bread and from 50% starch pastes show the same qualitative trends during staling, there are two outstanding

quantitative differences. The bread solubles are much greater in amount and substantially lower in iodine affinity than the solubles from a comparable starch paste. It seems probable that these differences are due to the influence of other bread constituents on the starch substance. Such hydrophobic polar materials as cyclohexanol or the higher fatty acids profoundly affect the pasting behavior of starch. These agents restrict granule swelling and likewise prevent leaching of the A-fraction. For example, if a dilute starch suspension is saturated with cyclohexanol and autoclaved at 123°C, the granules are only moderately swollen and no substantial amount of carbohydrate dissolves in the aqueous substrate. Similar effects are observed when starch is leached with water in the presence of excess fatty acid or cyclohexanol. The A-fraction is bound as an insoluble complex with the polar agent; the soluble extract is therefore small in amount and has an iodine affinity lower than that of the parent starch. Even the small amount (0.65%) of fatty acid normally present in corn starch raises the gelatinization temperature, restricts granule swelling, and impedes solution of the A-fraction. Only 5% of soluble amylose can be leached from nondefatted corn starch by water at 70°C (Kerr and Severson, 1943), as compared with 14% from defatted starch.

The use of simple wheat starch pastes permits evaluation of the separate influence of other bread constituents (Table III). The addition of 2% oleic acid markedly reduces the swelling power, amount of solubles, and iodine affinity of the solubles, even with freshly prepared pastes. It might be anticipated that a mono- or diglyceride would have a similar action. Shortening itself has little effect unless fatty acid develops by rancidification. The amount and iodine affinity of the soluble starch from bread must therefore be conditioned by natural

TABLE IV

COMPARATIVE EXTRACTION DATA FOR BREAD AND FOR MALTED STARCH PASTES (CALCULATED TO DRY STARCH BASIS)

	Age	Extraction at 30°C		Extraction at 50°C	
		Solubles	I_2 affinity	Solubles	I_2 affinity
	Days	%		%	
Bread[1]	0	9.6	2.1	11.9	2.9
	3	4.8	1.5	10.3	2.8
	6	4.1	1.4	10.5	2.7
Malted starch paste	0	10.9	1.6	11.7	1.9
	3	6.8	1.5	12.4	1.8
	6	4.0	1.7	9.8	1.7

[1] Data for bread averaged from Tables I and II and calculated to starch basis.

lipids of the wheat starch and by fatty acids developed in the shortening.

Natural or added enzymes in the flour may likewise have a modifying influence on the yield and character of the soluble starch. When malt extract is added to the starch prior to pasting, the amount and iodine affinity of the solubles closely parallel the values for bread. This similarity is particularly striking if the data for bread are calculated to a dry starch basis, assuming that the bread contains 75% starch (Table IV). Presumably the solubles are increased by alpha-amylase action during the early stages of baking.

Salt or yeast has little influence on the staling of 50% starch pastes. Some evidence of an insoluble starch-protein complex was anticipated, but neither casein nor egg albumen (exemplifying heat-stable and heat-coagulable proteins) has any very substantial effect on the staling of starch pastes. If anything, egg albumen appears to retard staling to a slight degree.

Aggregation of the B-Fraction of Starch

Oriented aggregation of the linear A-fraction is a well-established phenomenon, as shown by X-ray studies on the retrograded A-fraction and on stretched films of its acetate. Aggregation of the branched B-fraction is less clearly defined, but certain of the more obscure reactions of starch can perhaps be ascribed to such a cause. Meyer and Bernfeld (1940) have attributed the integrity of the granule and its pasting behavior to the interlocking structure of the amylopectin (or B-fraction), whereby the branches of neighboring molecules are organized into intermolecular areas of crystalline association ("fringe micelles"). The gradual relaxation of associative forces in these crystalline areas at elevated temperature is offered as explanation for the swelling of the granule. Sair and Fetzer (1944) have observed that potato starch undergoes a remarkable transformation when heated to 100°C in the presence of moderate amounts of moisture (i.e., 20–40% on dry starch basis). While the potato starch granule retains its birefringence under these conditions, its X-ray pattern passes from the B-pattern of a tuber starch to the A-pattern usually associated with the cereal starches. The gelatinization temperature is raised by this so-called "heat-moisture treatment," and the character of the paste more closely approaches that of the common cereal starches. It might be speculated that the branched B-fraction is present in the potato starch granule in less aggregated form than in the cereal starches, and that heating in the presence of limited amounts of water permits rearrangement into a more highly oriented association. A major difference between the tuber and cereal starches may arise from the fact that the

latter have been naturally "heat-moisture treated" during ripening of the grain. Observations in this laboratory show that the gelatinization temperature of immature (milk-stage) corn starch is some 8°–10°C lower than that of the mature corn starch. This might be interpreted to mean that the degree of association within the granule increases with maturation of the grain. MacMasters *et al.* (1946) have observed that when pastes of waxy maize starch are frozen and thawed, the starch loses its pastelike character and becomes insoluble at room temperature. From microscopic studies, they have further observed that this transformation appears to be accomplished through the formation of a "coacervate." Kruyt (1930) has applied the latter term to the separation of a colloid from solution as an emulsoid of liquid droplets that may subsequently coalesce to give a discrete layer. The decreased solubility of the starch coacervate suggests that some sort of intermolecular association has been established.

A further example of aggregation has been observed in this laboratory. When small amounts (10–20% by volume) of the lower alcohols are added with stirring to a 2–3% solution of corn B-fraction at room temperature, the solubility of the B-fraction is not markedly affected. Even after standing for several days at room temperature, the clarity of the mixture is not visibly different from that of the original solution of B-fraction. But if the mixture is refrigerated overnight at 3°–5°C, the B-fraction is quantitatively precipitated from solution. This precipitate is insoluble at room temperature and may be filtered on a Buchner and washed with water. The physical form of the precipitated B-fraction under the microscope depends on the amount and type of alcohol employed. Thus, 10% addition of methanol gives a microscopic floc, 20% methanol gives spherules approximating the size of the corn starch granule, and 30% gives small spherules the size of rice starch granules. Ethyl, propyl, isopropyl, sec.-butyl, isobutyl, tert.-butyl, and tert.-amyl alcohols all give microscopic flocs at concentrations from 10% to 20% by volume.[6] In no case does precipitation of the B-fraction occur at 5% concentration of any of these alcohols. Above 30% alcohol concentration, the precipitates begin to assume the doughy character of B-fraction when flocculated by gross addition of alcohol; this type of product can be dissolved in water at room temperature.

[6] This behavior has been utilized to effect a separation of the starch fractions by precipitation at different temperature levels. If a hot autoclaved starch solution is treated with 15–35% by volume of these alcohols (or if the starch is autoclaved in an aqueous alcohol medium of this concentration), the A-fraction may be separated by cooling to room temperature and supercentrifuging. If the centrifugate is then refrigerated to 3°–4°C, the B-fraction precipitates quantitatively. This separation is only effected if sufficient alcohol is employed so that the concentration of dissolved alcohol in the refrigerated solution of B-fraction is at least 10% by volume. Thus n-butyl alcohol cannot be employed, since it is not sufficiently soluble in water at 0°C to cause flocculation of the B-fraction. Obviously the alcohol concentration must not exceed 40% or the entire starch substance will be immediately precipitated. Preferred systems include 25% isopropyl alcohol and 18–20% sec.-butyl alcohol.

When these precipitates are slowly heated in water medium, their insolubility persists until the temperature reaches approximately 50°C, when the product tends to dissolve. This solubilization can be followed most readily under the microscope with the large spherules obtained by refrigeration with 20% methanol. If these are suspended in water and heated at a gradually rising temperature (most conveniently on the Kofler micro hot stage), there is no change in the size and shape of the spherules until the temperature reaches 52°C. At this point the spherules commence to swell, in a manner very similar to the swelling of a starch granule. Swelling is progressive up to 70°C, when the greatly swollen spherules begin to rupture and their outlines become vague. It should be remarked that no optical anisotropy has been observed with any of these B-fraction precipitates, either spherules or micro flocs. Through the courtesy of Dr. R. E. Rundle of Iowa State College, X-ray diffraction patterns have been obtained from the undried precipitates formed by refrigeration with 10%, 20%, and 30% additions of methanol. He reports that these products yield, respectively, a fairly prominent B-pattern, a faint B-pattern, and an amorphous diagram. Hence it appears that some sort of orderly crystallization of the B-fraction occurs, rendering that substance insoluble in water except at elevated temperatures. As an extension of these observations, the swelling behavior of starch must be attributed to organization within the granule and not to the presence of any exterior "membrane."

There would seem to be a close parallel between this type of crystallization and the staling of bread. Both reactions are most pronounced at temperatures around freezing, both give rise to a B-type X-ray pattern, both tend to reverse at temperatures above 50°C. As a tentative hypothesis, it is suggested that the following reactions typify aggregation of the branched B-fraction:

1. Staling of bread
2. Staling of 50% starch pastes
3. "Coacervation" by freezing
4. Precipitation by refrigeration with alcohol
5. Granular structure and pasting behavior of starches, especially those of waxy type

The primary conditions which seem to be requisite for this change are (1) a limited amount of water to permit orientation of the branches of the B-fraction and (2) relatively high concentrations of B-fraction. With respect to the first condition, it is known that crackers stale only when their moisture content becomes too high. Similarly, the starch

industry has repeatedly observed that pregelatinized starches dried on hot rolls tend to lose their cold water dispersibility (a "staling" reaction) when stored at high moisture levels. With respect to the second condition for aggregation, the B-fraction has gained its reputation for colloidal stability mainly on its behavior in dilute solutions. In each of the five instances cited above, the B-fraction either is in concentrated solution or else passes through such a phase. Thus, it is progressively deprived of water by freezing, it is laid down in the maturing grain at high concentrations, it appears to separate from water-alcohol medium as an emulsion of highly concentrated liquid droplets which then undergo crystallization. A starch coacervate might be regarded as a somewhat disorderly "brush-pile" of molecules, rendered insoluble by reason of molecular entanglement and associative cross-linkage. Crystallization of such a coacervate may occur if the colloidal substance has sufficient linearity and if conditions permit a certain freedom of orientation. An emulsion of lauryl alcohol in water provides an excellent analogy of this secondary crystallization. Rapid cooling gives a random crystallization of lauryl alcohol leaflets throughout the emulsion droplet. With slow cooling, the droplet is transformed into a perfect sphero-crystal, exhibiting a much more brilliant polarization cross than any starch granule.

Methylation analysis, as well as recent end-group assay by periodic acid oxidation, indicates the branch lengths of most of the common B-fractions to be of the order of 20-30 glucose units. This would therefore constitute the effective branch length undergoing this type of aggregation. On this basis, retrogradation of the A-fraction differs only by virtue of its much longer chain length (400–1000 glucose units) and the consequent higher order of association. In contrast, animal glycogen is reputed to be less susceptible to insolubilization, in agreement with its estimated branch length (from end-group assay) of only 11 glucose units (Meyer and Fuld, 1941). The so-called "glycogen" from sweet corn (Morris and Morris, 1939) does not possess sufficient associative tendency to permit its organization into granular form. When a solution of this "corn glycogen" is frozen and then allowed to thaw, it shows no tendency to become insoluble. Similarly, the present writers have observed that the beta-amylase limit dextrin from waxy maize starch [7] is not insolubilized either by freezing or by refrigeration with 10–20% methanol. The same is true of the low-substituted ethyl and hydroxyethyl ethers of starch and (to a lesser degree) of commercial hypochlorite-oxidized corn starches. Presumably the side-branches of these products have been removed or

[7] The limit dextrin was furnished through the courtesy of Miss Edna Montgomery, Northern Regional Research Laboratory, Peoria, Illinois.

else so warped by chemical modification that they are no longer capable of side-by-side association.

It is conceivable that such products (especially the beta-amylase limit dextrin) may have practical utility as antistaling agents for bread. This would perhaps depend on the site of staling in the crumb. It would be difficult to prevent staling within the individual swollen granules by the addition of any such adjunct, since the latter could not penetrate into the gel lattice of the swollen granule. However, the use of such adjuncts in relatively small proportions might prevent cementing between the granules, which may be of even greater importance in the consumer's evaluation of staling. It should be recalled that the latter does not correspond with chemical criteria of staleness. The consumer generally considers 2-day old bread as fairly fresh, though swelling power and percentage solubles would rate such bread as highly staled.

The authors wish to express their thanks to Dr. W. F. Geddes for his helpful comment and advice during the course of this work.

Summary

The water-soluble starch leached from the crumb of fresh bread at 30°C is predominantly the branched B-fraction, as evaluated by potentiometric iodine titration. The linear A-fraction of the starch must be insolubilized by retrogradation during baking and hence cannot contribute to the staling process. The progressive decline in solubles during the aging of bread indicates that the cause of staling may be a spontaneous aggregation of the branched B-fraction. As additional evidence for this theory, the percentage of soluble B-fraction from staled bread is restored to a constant high level when leached at 50°C; this is in accord with the refreshening of bread by moderate heating. No substantial solubilization of the A-fraction occurs at 50°C.

Parallel behavior is observed during the aging of simple 50% aqueous pastes of defatted wheat starch, prepared under conditions to simulate the baking of bread. This further substantiates the theory that staling is inherent in the B-fraction, and not due to interaction between the starch and other bread ingredients. However, the behavior of the starch substance in bread and the nature of the staling action may be considerably influenced by the presence of other bread constituents. This is shown by a study of the solubles leached from simple starch pastes prepared with the incorporation of various individual bread constituents. Thus malt extract greatly increases the amount of soluble starch and decreases its iodine affinity; free fatty

acid depresses both the amount and iodine affinity of the solubles. Normal amounts of salt, yeast, neutral shortening, casein, or egg albumen have little influence on the staling of 50% starch pastes. It is assumed that these latter constituents will play a minor role in bread staling.

Pure B-fraction undergoes aggregation effects which may typify its behavior in bread staling. If 10–30% alcohol is added to a solution of B-fraction and the mixture refrigerated, the B-fraction precipitates as a floc or as minute spherules, insoluble in water at room temperature and possessing a B-type X-ray pattern. The spherules commence to gelatinize in water at 52°C, undergoing much the same swelling as natural starch granules. It is suggested that the various aggregation phenomena of the B-fraction (including staling) represent an oriented association between the branched chains of the B-fraction molecules. No such aggregation is observed when these branches are removed or modified, as with the beta-amylase limit dextrin, water-soluble starch ethers, and hypochlorite-oxidized starches.

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STARCH GELATINIZATION STUDIES. I. SIMPLIFIED EQUIPMENT FOR THE STUDY OF STARCH GELATINIZATION BY MEANS OF LIGHT TRANSMISSION [1]

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(Received for publication March 3, 1947)

There are a number of methods available for the study of starch gelatinization. These have been reviewed by Radley (1943). A method that appears to be simple and easily controlled, and that also gives excellent replication of results, is the one based on the measurement of light transmission through the gelatinizing starch dispersion (Cook and Axtmayer, 1937). The equipment used by Cook and Axtmayer was improved by Küntzel and Doehner (1939) and by Morgan (1940). However, these were special setups of a light source and a photoelectric cell. Since apparatus for measuring light transmission (the spectrophotometer) is common laboratory equipment, it seemed that the study of gelatinization by means of light transmission could be considerably simplified by adaptation to this equipment. The present paper describes one such adaptation which has been found convenient for the study of starch gelatinization in this laboratory.

Equipment and Method of Operation

The spectrophotometer used for this work was a Coleman Model 11.[2] The cuvette carrier, originally designed to hold two cuvettes, was adapted to carry a U tube by removing the partition between the two cuvette openings.

The gelatinizing equipment consists of a U tube (with dimensions as shown in Figure 1) with a bridge (A) between the two arms. The arms are wrapped (as shown in Figure 1) with 160 cm of B and S No. 26 Chromel resistance wire and then insulated by wrapping with asbestos cord. A screw-type glass stirrer is used in one arm of the U tube and a thermometer in the other.

When in operation, the stirrer is turned by a variable speed motor at a rate which circulates the liquid rapidly enough to keep un-

[1] Published with the approval of the Director as paper No. 417 Journal Series, Nebraska Agricultural Experiment Station. The subject matter of this paper has been undertaken in cooperation with the Committee on Food Research of the Quartermaster Food and Container Institute for the Armed Forces. The opinions or conclusions contained in this report are those of the authors. They are not to be construed as necessarily reflecting the views or indorsement of the War Department.

[2] It is recommended that the spectrophotometer be equipped with a constant voltage regulator for direct line operation. Both the spectrophotometer and voltage regulator should be grounded. If a battery is used, it must be in first-class condition since the galvanometer can be adjusted only at the beginning of a 35-minute run; the light intensity must stay constant during this period.

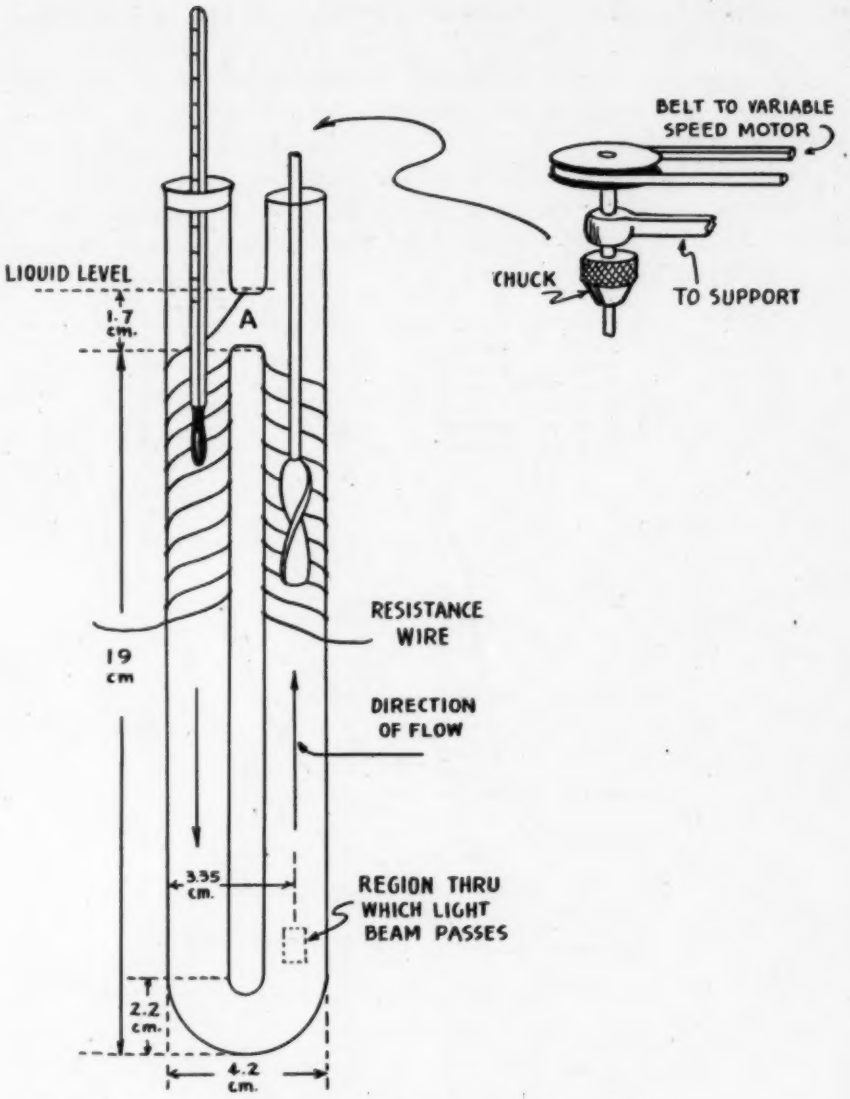

Fig. 1. U tube equipped for the study of gelatinization by means of light transmission.

gelatinized starch in suspension. However, the stirring should not be vigorous enough to cause air bubbles to be entrapped and circulated. By circulating the liquid upward in the arm through which the spectrophotometer light beam passes (as indicated by the arrows in Figure 1), the entrapment of air is avoided.

Rate of heating is controlled by a variable transformer. Morgan (1940) used a rate of 2.5° per minute. Cook and Axtmayer suggested

that a slower rate (1.5 to 2.0 minutes for each degree C rise in temperature) was preferable.

For light transmission studies, the ungelatinized starch suspension must be dilute enough to give some transmission of the light beam so that the first changes in gelatinization are observable. Cook and Axtmayer added suspended starch to water in the reaction vessel until the microammeter gave a reading of 3 to 5 microamperes. Morgan used 0.5% dispersions which, with his equipment, gave readings between 5 and 15 microamperes.

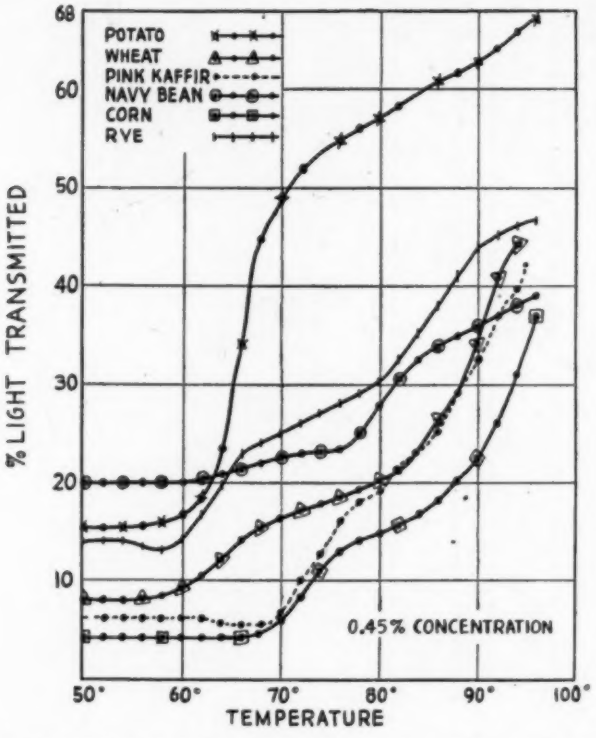

Fig. 2. Light transmission curves of 0.45% concentrations of some common starches.

In operation, the U tube is filled with enough water just to cover the bridge (A of Figure 1) [3] and is placed in position in the spectrophotometer. The stirrer is started and the galvanometer is adjusted to read 100% light transmission. Dry starch is introduced through a funnel onto the surface of the circulating liquid. As soon as the galvanometer gives a constant reading for percent light transmission, indicating complete dispersion of the starch, heating is started and maintained at the desired rate. Light transmission readings may be

[3] The tubes described in this paper require 66 ml.

made at any desired interval; at critical periods the readings should be frequent.

Discussion of Light Transmission Curves

Figure 2 shows gelatinization curves obtained from 0.45% dispersions of some common starches heated at a rate of 2°C per minute. These curves are similar to those shown by Cook and Axtmayer (1937) and by Morgan (1940). They show the wide variation in gelatinization characteristics which is found among different starches.

The Effect of Varying Starch Concentration. Figure 3 shows gelatinization curves for wheat starch obtained by heating three concentra-

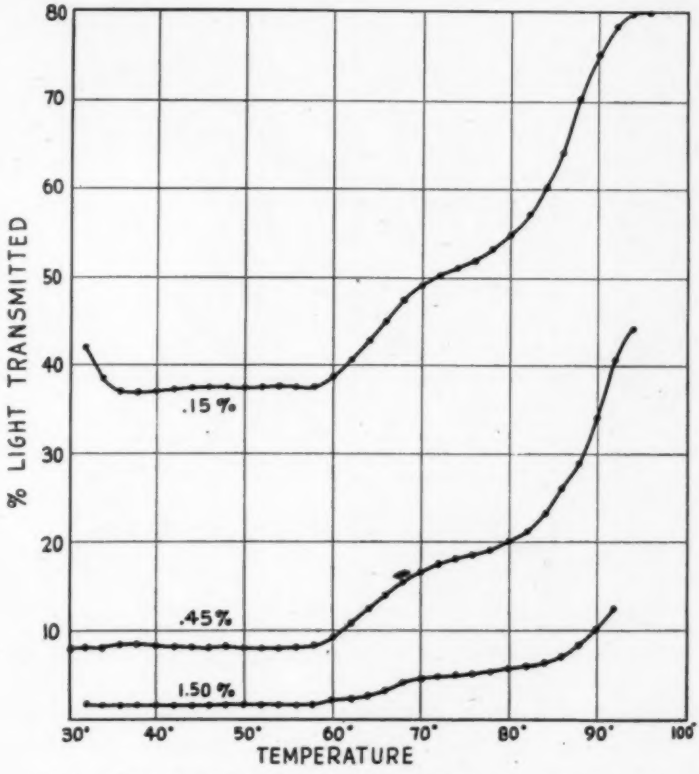

Fig. 3. Effect of wheat starch concentration on light transmission curves.

tions of wheat starch at the rate of 2°C per minute. These data indicate that the gelatinizing characteristics of starch are more clearly shown by the use of the lower concentrations. Since spectrophotometer determinations are most accurate on solutions giving 10% to 60% light transmission, it is well to use a concentration of starch which gives curves falling largely within these limits.

Figure 4 shows data obtained on the same starches as shown in Figure 2, but using 0.20% dispersions. The curves of Figures 2 and 4 are similar in character; however, the curves of Figure 4 give more detail and emphasize the differences among the starches before gelatinization and in the early stages of gelatinization.

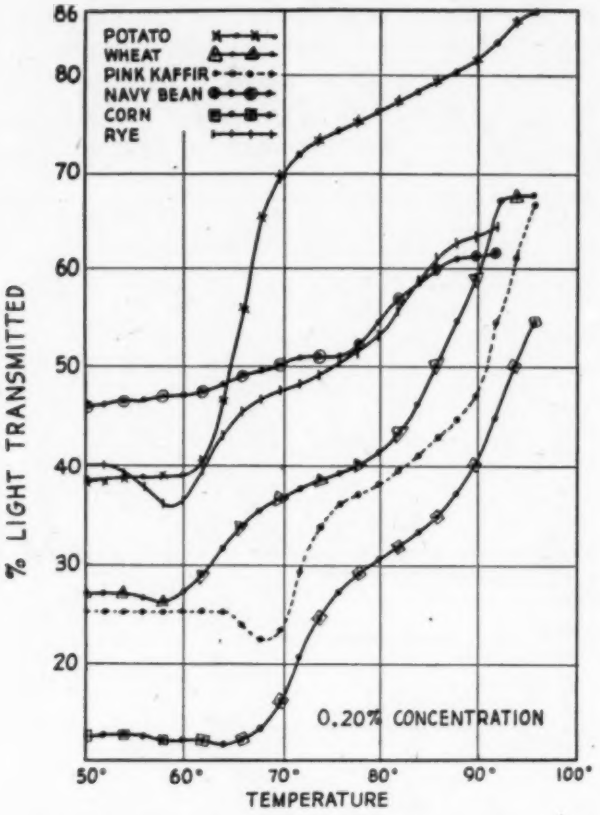

Fig. 4. Light transmission curves of 0.20% concentrations of starches. Compare with Figure 2.

The Effect of Varying the Rate of Heating. Cook and Axtmayer (1937) state that the rate of heating affects the "temperature of transition" (the first indication of gelatinization) but does not change the slope of the curve. They found the temperature of transition of cassava starch to be shifted 3°C higher by increasing the rate of heating from 0.16°C to 1.25°C per minute. However, in this laboratory, using the equipment as described, wheat and corn starch gelatinization curves have given excellent replication even when the rate of heating has been varied from 2.5°C to 0.15°C per minute. No significant change could be noted in the "temperature of transition."

Differences in light transmission among native unaltered starches at temperatures below those at which gelatinization begins are due to differences in granule (or aggregate) size (Morgan, 1940); the smaller granules give less light transmission. A decrease in light transmission at the beginning of a curve, as in the upper curve of Figure 3, is due to incomplete dispersion; whereas a dip in the curve at the beginning of gelatinization, as shown by the kaffir and rye starches in Figure 4, may be attributed to disintegration of aggregates of granules (Morgan, 1940). Unpublished work, using other methods of studying gelatinization, indicates that the beginning of the dip coincides with the beginning of gelatinization.

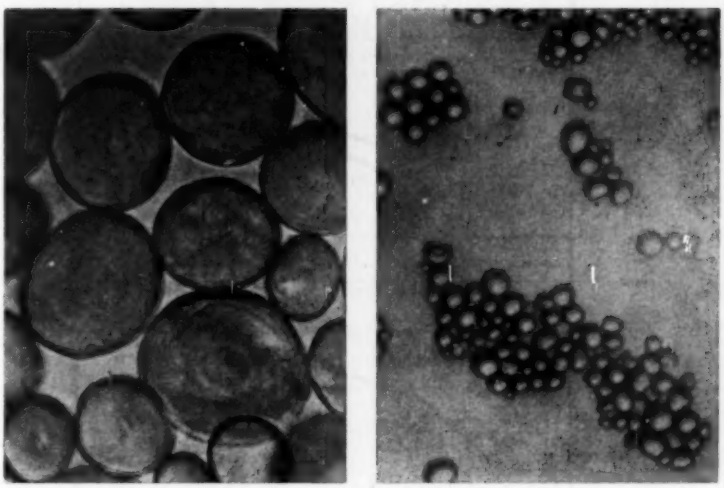

Fig. 5. Preparations of large and small granule wheat starch (× 600).

Alsberg and Rask (1924) showed by viscometric methods that gelatinization of wheat and maize starches was a gradual change taking place over a range of 25° to 30°. They pointed out that if the temperature of gelatinization were defined as that at which anisotropy disappears, it must be regarded only as an early stage of the entire gelatinization process. Light transmission curves not only lend emphasis to these observations but the change in rate of gelatinization at certain temperatures suggests that gelatinization takes place in steps (see the curves of wheat, corn, rye, and kaffir, Figure 4). Katz (1928) stated that "for a long time authors have described two grades or stages of gelatinization." Steps in the gelatinization process as shown in Figure 4 correspond to Katz' grades. The first step corresponds to the generally accepted gelatinization temperature (variously defined as that temperature at which the granules swell, begin to lose

starch to the solvent, lose their anisotropy, lose their resistance to staining by certain dyes, etc.), whereas with some starches the second step takes place at considerably higher temperatures.

Morgan (1940) suggested that the changes in gelatinization rate occurring at certain temperatures, as shown by his gelatinization curve of wheat starch, represented the gelatinizing temperatures respectively

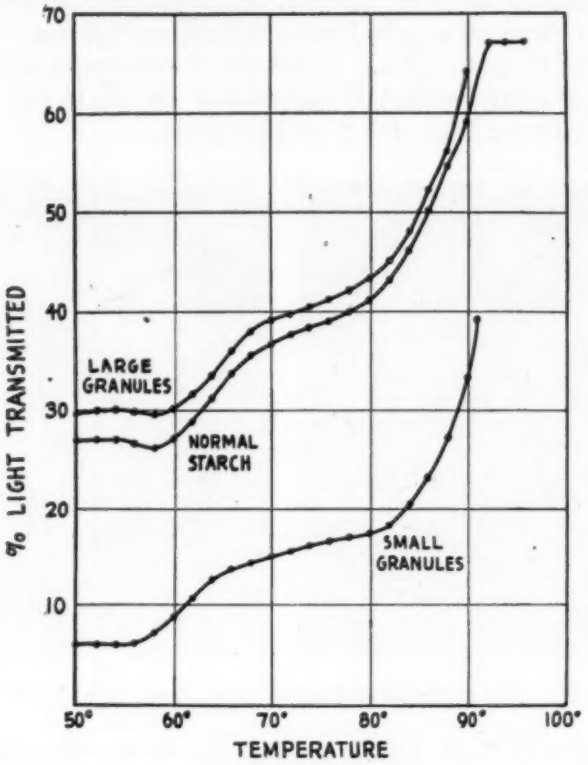

Fig. 6. Effect of size of granule on the light transmission curves of wheat starch.

of the large and small granules. Since the large and small granules may be separated by repeated centrifuging, this supposition may be readily checked.[4]

The photomicrographs in Figure 5 show the character of the large and small granule wheat starch preparations separated by centrifuging. A comparison of the light transmission curve of the original mixture

[4] It is exceedingly difficult to free the small starch granules from the nongranular gelatinous material which consists largely of the dextrins resulting from beta-amylase action on starch damaged in the milling process. This has led some investigators to attribute properties to the small granule starch (MacMasters and Hilbert, 1944) which our data indicate are due to nongranular contaminants. In this laboratory the best small granule starch preparations have been obtained by first centrifuging out and discarding the "amylodextrin" fraction. This results in the loss of much small granule starch; however, it is difficult to purify satisfactorily this starch, not only from the gelatinous contaminants but also from fragments of large granules. The mixture of small and large granules, freed from the gelatinous material, is then subjected to repeated centrifuging to obtain the two types of starch.

of wheat starch granules with curves for the small and large granule preparations is given in Figure 6. These data indicate that the shape of the curve is not determined by the presence of a mixture of various-sized granules in the preparation but is dependent on differences in the character of the intermolecular bonds within the granule.

It should be emphasized that the steps or stages in gelatinization as indicated by the gelatinization curves are not sharp transitions occurring at one temperature, but each is a gradual transition covering a range of 10°–15°C, and may be further subdivided. An entire step or stage cannot be passed through by holding the temperature constant

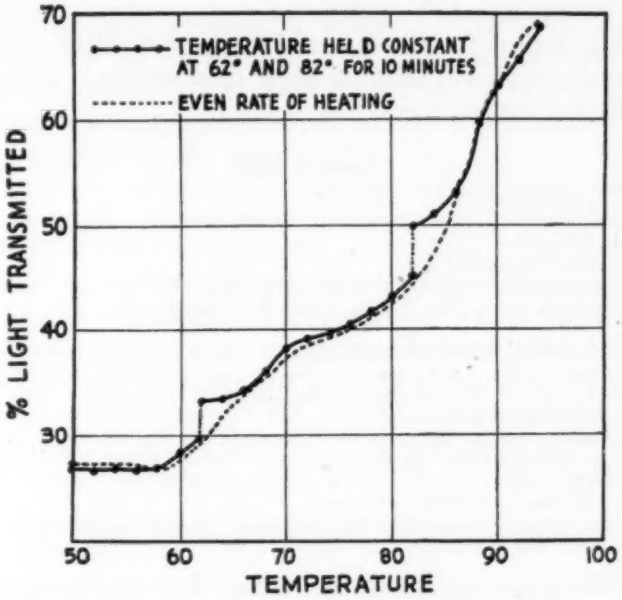

Fig. 7. Effect of holding the temperature constant for a period of time during the gelatinization procedure.

at the beginning of the step, e.g., the first step in the gelatinization of wheat starch occurring between the temperatures of 55°C and 70°C cannot be completed by holding the temperature constant at 55°, at 60°, or even at 65°. This is illustrated in Figure 7. However, it is to be noted that an increase in light transmission does occur on holding the temperature constant for a time. This indicates that the curve might be considerably altered by exceedingly slow heating, although, as before stated, the curves for wheat and corn starches were not changed by varying the rate of heating from one requiring less than 30 minutes to one requiring over 4½ hours for a curve complete from 30° to 95°.

Summary

Equipment was developed for adapting the spectrophotometer to the study of starch gelatinization.

It was found that within the limits of from 2.5°C to 0.15°C per minute, the rate of heating had no effect on the curve characteristics nor on the gelatinization temperatures of wheat and corn starches.

The size of the starch granule determined the amount of light transmitted by ungelatinized starch suspensions. Large and small granule wheat starch preparations gave curves essentially similar in character, indicating that the two types of wheat starch granules are similar in gelatinization characteristics.

The data confirm the statement of Katz (1928) that gelatinization takes place in steps and that for some starches these steps occur at widely differing temperatures.

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METHODS FOR DETERMINATION OF ALPHA-AMYLASE. IV. A GLASS END POINT COLOR STANDARD FOR USE IN THE DEXTRINIZING METHOD; EFFECT OF TEMPERATURE AND STARCH LOT ON THIS METHOD

SUTTON REDFERN

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(Presented at the Annual Meeting, May 1946; received for publication May 5, 1947)

The Wohlgemuth procedure developed by Sandstedt, Kneen, and Blish (1939) for the determination of alpha-amylase has achieved widespread popularity. The method promises to become even more widely used now that a commercial source of pure beta-amylase is available for preparing the substrate.

In this method, alpha-amylase activity is expressed in terms of the digestion time required for the enzyme to convert starch to products which give a red-brown coloration with iodine. The standard end point is the color produced by a specified combination of Merck's Reagent dextrin and iodine. This end point is an improvement both over the previously used erythropoint at which the color changes from blue to red, and over the achromic point at which the color disappears. However, no critical study of the dextrin-iodine end point color has been reported. It is one purpose of this paper to describe a new permanent glass color standard to replace the dextrin-iodine standard.

Comparison of Different Lots of Dextrin

Dextrins are degradation products of starch. It was thus expected that different lots of dextrin would vary with respect to their iodine color. To substantiate this, spectrophotometric data for five different lots of Merck's Reagent dextrin were obtained with a Beckman quartz spectrophotometer using 1 cm cells and a water reference blank. Transmittancy values were measured in 10 mμ steps. The dextrin-iodine color was prepared as described by Sandstedt, Kneen, and Blish using a weight of dextrin equivalent to 0.528 g dry dextrin per liter of water.

In Figure 1, the transmittancy values for each dextrin-iodine color are plotted against wavelengths of light. Because of the presence of excess iodine, all of the dextrin-iodine solutions completely absorbed all light below a 500 mμ wavelength. The curves show wide variations in the color produced by each dextrin. Moreover, the crossing of the curves for dextrins Nos. 1 and 2 even indicated differences in color

hues, since the solutions were of equal optical density, as will later be shown in Table I.

The curves in Figure 1 are not sufficient to define the quantitative effect on the dextrinizing method of the variations in dextrin lots. Therefore, each dextrin-iodine color was used as the end point for determining the dextrinizing time of a standard barley malt sample. Total light transmittance, expressed as optical density, was measured

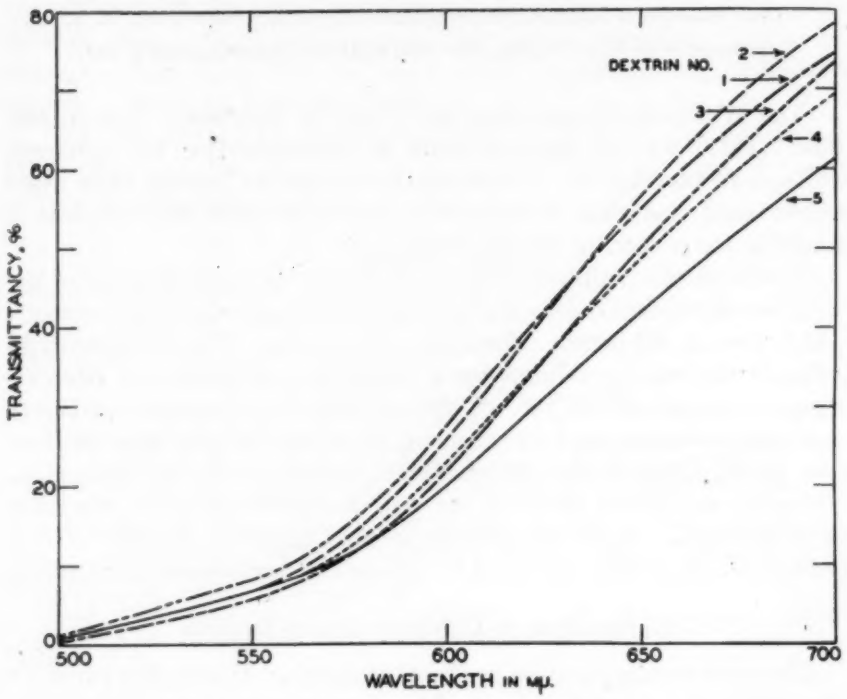

Fig. 1. Transmittancy curves for dextrin-iodine solutions.

with an Evelyn colorimeter without a filter. The hydrolysis rate of the beta-amylase starch substrate was followed by measuring the transmittancy of the hydrolyzate-iodine color at appropriate time intervals with the Evelyn colorimeter. By plotting transmittancy against reaction time, the dextrinizing time corresponding to the optical density of each dextrin-iodine solution was determined. The dextrinizing time was also determined visually using each dextrin-iodine color in a Hellige comparator.

The optical density of each dextrin-iodine color and the dextrinizing times for each as determined by the Evelyn colorimeter and the Hellige comparator are given in Table I. The dextrinizing times determined by the Evelyn colorimeter varied from 15.75 to 19.75 minutes,

a range of four minutes or about 22% of the average. In the Hellige comparator, the times varied from 17.25 to 19.0 minutes, a range of 1.75 minutes or about 10% of the average. Since it is easily possible to have duplicate analyses agree within 0.25 minute, the differences in dextrinizing times obtained with either method are much too large to be neglected.

TABLE I

EFFECT OF DIFFERENT LOTS OF MERCK'S REAGENT DEXTRIN ON DEXTRINIZING TIME

Dextrin no.	Optical density	Dextrinizing time	
		Evelyn colorimeter	Hellige comparator
		Min.	*Min.*
1	0.463	19.75	19.0
2	0.464	19.75	18.5
3[1]	0.511	18.25	18.0
4	0.517	18.0	18.0
5	0.600	15.75	17.25

[1] Sample used for 1946 collaborative study by Malt Evaluation Committee, A. A. C. C.

The differences between dextrinizing times as determined by the Evelyn colorimeter and by the visual comparator are due, most probably, to the differing response of the eye and the photocell, and to the fact that the hue of the dextrin-iodine color does not exactly match that of the hydrolyzate-iodine color at the end point.

The dextrin-iodine color standard also has other disadvantages. Neither stock dextrin nor dextrin-iodine solutions are stable. Kneen (1946) recommended that a fresh dextrin-iodine solution be prepared daily and a fresh dextrin stock solution monthly. It has also been this author's experience that the hue of the dextrin-iodine color is not the same as the hue of the hydrolyzate-iodine color, at least when they are compared at a solution depth of 13 mm. This makes it difficult to select the exact end point.

Inorganic Color Standards

A permanent color standard would be very desirable. Redfern and Landis (1946) and Olson, Evans, and Dickson (1944) have described inorganic standards made from cobalt chloride, potassium dichromate, and hydrochloric acid, which differ only in the proportions of each component. Neither of these standards exactly matched the hue of the dextrin-iodine color. The solutions were not spectrophotometrically equivalent, so that the color match was only visual. This may be shown by comparing the transmittancy curve for the Landis-Redfern color standard in Figure 2 with the transmittancy

curves for the dextrin-iodine colors in Figure 1. It should be noted that visual match is dependent upon viewing depth, since both the inorganic color solutions and the dextrin-iodine solution are dichroic, i.e., different thicknesses exhibit different hues.

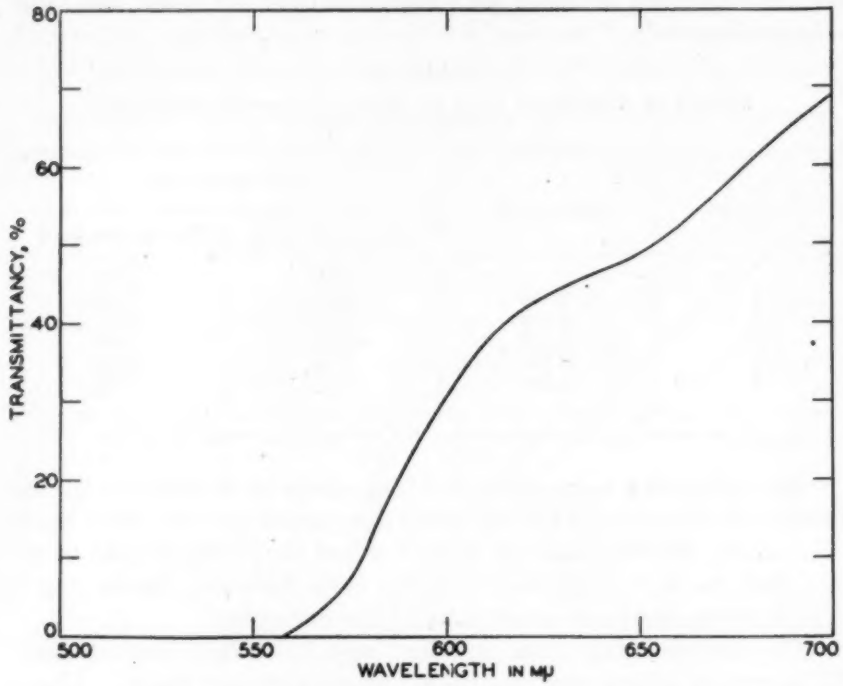

Fig. 2. Transmittancy curve for Landis-Redfern inorganic color standard.

In an attempt to find one combination of inorganic salts that was spectrophotometrically equivalent to the dextrin-iodine color, a study was made of various combinations of cobalt sulfate, cobalt chloride, ferric chloride, copper sulfate, potassium dichromate, uranyl acetate, and potassium chloroplatinate. No such universal combination was found. Each depth of the dextrin-iodine color required a different mixture of the inorganic salts.

Hellige Varnish Color Standard

A permanent glass color standard was sought, which would be non-fading, stable, and always ready for use. It was found that the No. 17 Hellige varnish color is a very close visual color match to the dextrin-iodine color at a depth of 13 mm. This varnish color is one of the 18 standard varnish colors of the Institute of Paint and Varnish Research, and, therefore, has the advantage of being available as a

stock item. A comparison of five different No. 17 varnish colors showed deviations between them no greater than 2%. This accuracy should be compared with the 25% difference shown in Table I between the optical density extremes of the iodine colors from different lots of dextrin.

The No. 17 varnish color has the further advantage of matching the hue of the hydrolyzate-iodine color much better than does the dextrin-iodine color. The importance of this factor is readily apparent.

Hellige Varnish Color Standard and Comparator Applied to Dextrinizing Method

The No. 17 varnish color standard can be used in either the standard Hellige comparator (607) [1] or the pocket comparator with prism attachment (605-A).[1] The regular varnish color disc contains a series of colors from Nos. 11 to 18, but a special alpha-amylase color disc (620-S5) [1] containing only the No. 17 color may be obtained at a lower cost than the complete disc. This special disc is the preferred color standard because the manufacturing tolerance is much smaller than that permitted for the same color in the regular varnish color disc. The center of the complete varnish color disc normally contains a light green glass filter which must be removed. The special alpha-amylase color disc does not have this filter.

The comparator is conveniently illuminated with a 100-watt frosted bulb. Comparison of the hydrolyzate-iodine color with the No. 17 varnish color involves use of a square tube of 13 mm viewing depth which is standard equipment with the Hellige comparator. The square tube is recommended in preference to round test tubes which must be selected to have a 13 mm viewing depth.

The dextrinizing method makes use of the No. 17 varnish color and Hellige comparator as follows. The substrate and reagents are prepared according to the directions of Sandstedt, Kneen, and Blish (1939). A series of 13 × 100 mm test tubes containing 5 ml of dilute iodine solution "B" is prepared and attemperated at 30°C in readiness for testing. As the end point is approached, 1 ml of the hydrolyzing mixture is added to 5 ml of iodine solution and the hydrolyzate-iodine solution is poured into the 13 mm square tube for color comparison with the No. 17 varnish color. After the color comparison is made, the solution is poured out by giving the tube a quick shake. In this way, very little liquid remains in the tube and it is ready for another test. It is the author's custom to remove a sample every 0.5 minute on the minute or half-minute. In case two samples 0.5 minute apart show

[1] Catalog numbers of Hellige, Inc., Long Island City, New York, N. Y.

that one is darker and the other lighter than the No. 17 color standard, the dextrinizing time is interpolated as the quarter minute between these two times.

Since the dextrin-iodine color standard varies with the dextrin batch, it is proposed that the No. 17 varnish color be accepted as the standard end point color when the hydrolyzate-iodine color is viewed in a 13 mm square tube.

Kneen and Sandstedt (1941) published a method for the determination of beta-amylase in which the saccharification due to the alpha-amylase was subtracted from the total saccharifying activity. A table relating alpha-dextrinogenic activity to alpha-saccharogenic activity was compiled, but this table is only valid for alpha-amylase activities determined with the aid of the particular dextrin sample used by these authors. It cannot be used by other workers using a different dextrin without introducing a possible error. The magnitude of this error has not as yet been determined, but it will be necessary to re-examine the relationship between alpha-dextrinogenic and alpha-saccharogenic activity using the new color standard. This same consideration applies to the work of Olson, Evans, and Dickson (1944).

Effect of Temperature on Iodine Colors

Although it is a well-known fact that the familiar blue starch-iodine color disappears when heat is applied (Kerr, 1944), the effect of temperature on the hydrolyzate-iodine and dextrin-iodine colors has not previously been reported. For the determination of temperature effect, a dextrin-iodine color standard using dextrin No. 3, and a hydrolyzate-iodine color which corresponded approximately to the end point were prepared. Each solution was adjusted to the desired temperature, and the total transmittancy was measured with an Evelyn colorimeter without a filter. The results are shown in Figure 3. Surprisingly, the dextrin-iodine color is nearly independent of temperature in the range from 15° to 30°C. The hydrolyzate-iodine color, on the contrary, has a larger temperature coefficient. The difference in optical densities at 20° and 30°C amounts to approximately 8%. In practice this temperature effect cannot be neglected.

The actual effect of temperature on dextrinizing time was determined by running duplicate hydrolyses using the same quantity of barley malt and the same substrate at 30°C. In one case the iodine solution was attemperated at 20°C and in the other at 30°C. The dextrinizing times for each temperature using the No. 17 varnish color as an end point are given in Table II.

The dextrinizing times differ by nearly 10% and this cannot be viewed with equanimity. The temperature of the hydrolyzate-iodine

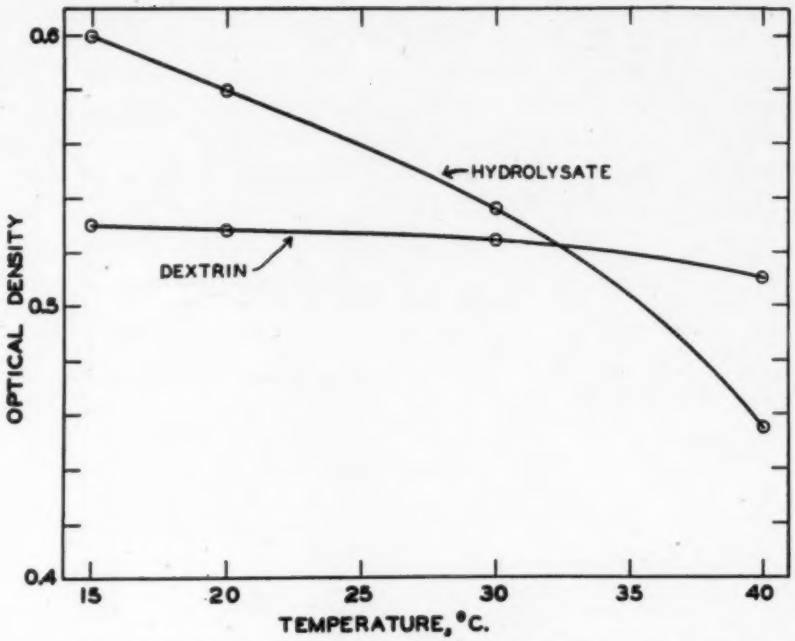

Fig. 3. Effect of temperature on dextrin-iodine and hydrolyzate-iodine solutions.

TABLE II

EFFECT OF TEMPERATURE OF IODINE SOLUTION ON DEXTRINIZING TIME

Temperature of iodine solution	Dextrinizing time	Alpha-amylase units (SKB)
°C	*Min.*	
20	18.75	32.0
30	17.0	35.3

color must be controlled. This is most easily done by attemperating the test tubes containing the iodine solution in the same 30°C water bath used for the digestion. It is true that the temperature may change slightly during the time required to make the color comparison, but this change will be practically constant.

Effect of Starch on Dextrinizing Method

For the preparation of the beta-amylase starch substrate, Sandstedt, Kneen, and Blish (1939) originally specified Merck's soluble Lintner starch. This was later changed to Merck's soluble Lintner starch, special for diastatic power determination (Kneen, 1946). The latter is the one approved by the American Society of Brewing Chemists for use in the determination of diastatic power. However, the

A. S. B. C. (1944) also approved the use of the soluble Lintner starch made by the J. T. Baker Chemical Co. Several different lots of soluble starch from each of the two companies were compared by preparing a substrate from each starch using Wallerstein beta-amylase and determining the dextrinizing time of a standard quantity of malt syrup acting on these substrates. The results in terms of alpha-amylase units are given in Table III.

TABLE III

EFFECT OF DIFFERENT LOTS OF SOLUBLE STARCH ON THE DEXTRINIZING METHOD

Description of soluble starch		Alpha-amylase units
Merck's lot no.	40515	75.4
	40455	75.7
	71741	76.0
	111243 } Different bottles	76.0
	111243 } Different bottles	76.8
	11446[1]	78.4
	4445[1]	78.8
Baker's lot no.	7344	80.5
	51045	81.8
	32743	81.8
	72045[1]	82.7
	102142	85.0
	121043	85.4

[1] Approved by the American Society of Brewing Chemists for diastatic power determination.

Merck and Baker starches fall into two distinct groups, with the Baker starches giving consistently higher values. The number of alpha-amylase units (SKB) for Merck starches varies from 75.4 to 78.8 with an average value of 76.7 units, and the Baker starches range from 80.5 to 85.4 with an average value of 82.9 units. Thus, the extreme variation of all the starches tested amounted to 10.0 alpha-amylase units (SKB), or approximately 12.5%. The average values for the approved Merck and Baker starches differed by about 5%. This is a definite difference which cannot be ignored.

Differences in starch lots are not unexpected and should not be construed as a criticism of either manufacturer. Approval of certain of these starches applies only for use in the official diastatic power method; they were not standardized for use in the dextrinizing method. However, there is evidently a fundamental difference in the preparation of these starches by each manufacturer and it is unfortunate that the starches are not interchangeable for use with the two methods. Hence, it is probable that the starch will have to be standardized for use in the dextrinizing method the same as is done for the diastatic power method, though it would be preferable to have one starch that could be used for both methods.

Summary

The effect of different lots of Merck's Reagent dextrin on the dextrin-iodine end point color standard used in the method of Sandstedt, Kneen, and Blish for the determination of alpha-amylase was studied. Different dextrins gave different spectrophotometric curves and when used as end points resulted in dextrinizing time differences ranging from about 10 to 20%.

It was found that the No. 17 Hellige glass varnish color standard when used with a Hellige comparator can replace the dextrin-iodine color standard with considerable advantage. The glass standard is permanent, reproducible, commercially available, and independent of temperature. The application of the varnish color standard to the dextrinizing method is herein described. This new glass standard is proposed as a replacement for the dextrin-iodine standard.

The dextrin-iodine color was found to be nearly independent of temperature, but the hydrolyzate-iodine color varies considerably with temperature. The dextrinizing time of a malt was significantly shorter when the temperature of the iodine solution was 30°C than when the temperature was 20°C. It is recommended that the dilute iodine solution be attemperated at 30°C before use to insure a standard temperature of testing.

Different lots of Lintner soluble starch showed a maximum variation of approximately 12.5% in alpha-amylase values obtained with the same malt, although starches approved for the diastatic power method differed by approximately 5%. There is a significant difference between soluble starches made by Merck and by Baker, the Baker starches giving higher alpha-amylase values.

Acknowledgment

Grateful acknowledgment is made to Dr. Allan D. Dickson for supplying samples of different lots of soluble starch, to the Merck Company for supplying known samples of Reagent Dextrin, and to the J. T. Baker Chemical Company for supplying samples of soluble starch.

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B-COMPLEX VITAMINS IN GRAIN SORGHUMS

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Grain sorghums are grown extensively in those areas of the Southwest where climatic conditions make corn production hazardous. Because of their ability to withstand high-temperature and low-moisture conditions, they have become an important crop in Texas, Kansas, Oklahoma, California, New Mexico, Nebraska, and Colorado.

During the decade 1930–1939, production of grain sorghums in the United States averaged about 52 million bushels annually. Owing to the development of dwarf types of sorghum suitable for machine harvesting and adapted to climatic conditions in this country, production has increased greatly. In 1944 about 182 million bushels were harvested from an estimated 9.1 million acres. Almost an equivalent acreage was planted for forage and silage.

Information concerning the production, composition, and uses of sorghums has been compiled by Edwards and Curtis (1943). As with corn, approximately 85% of the sorghum crop is used on farms. In view of this extensive use for feeding purposes and the limited data at hand concerning the vitamin content of this cereal, further information seemed desirable. Such data should allow better estimation of the vitamin contents of some derivatives and by-products resulting from the use of sorghums in industries such as distilling and milling.

The vitamin content of sorghums has received little attention. By a rat-growth method, yellow corn was reported (Smith, 1930) to have about 10 times as much vitamin A activity as yellow milo sorghum and about 20 times as much as the Hegari variety. Nordgren and Andrews (1941) reported that thiamine in seven unidentified sorghums varied from 1.93 to 3.97 mg per pound and averaged 2.68 mg per pound

[1] One of the laboratories of the Bureau of Agriculture and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

(4.75, 8.75, and 5.9 μg per gram respectively). Riboflavin, nicotinic acid, and pantothenic acid contents of 29 varieties of grain sorghum grown in 1941 and 1942 at Perkins and Woodward, Oklahoma, have been reported by Knox *et al.* (1944). Riboflavin varied from 1.17 to 2.08 μg per gram, pantothenic acid from 12 to 15 μg per gram, and nicotinic acid from 22 to 78 μg per gram.

In this report values are given for riboflavin, nicotinic acid, pantothenic acid, pyridoxine, and biotin contents of 42 sorghum strains. Samples were obtained from several locations through the cooperation of the Bureau of Plant Industry, Soils, and Agricultural Engineering.

Methods

All vitamin analyses of finely ground sorghum grain were made by microbiological methods. Riboflavin was determined by the Snell and Strong method (1939) following hydrolysis of the sample in 0.1 *N* hydrochloric acid for 30 minutes at 120°C, filtration after adjustment to pH 4.5, and, finally, neutralization to pH 6.8. Nicotinic acid determinations were by the method of Snell and Wright (1941) after hydrolysis of samples in 1 *N* sulfuric acid for 30 minutes at 120°C and filtration after neutralization to pH 6.8. Pantothenic acid was determined with *Lactobacillus arabinosus* (Skeggs and Wright, 1944). For this determination samples were steamed for 15 minutes, cooled, and digested for 16 to 20 hours at 37°C with a mixture of papain and clarase suspended in an acetate buffer, pH 4.6. Biotin and pyridoxine were liberated by autoclaving at 120°C for 30 minutes in the presence of 5 *N* sulfuric acid; biotin was determined in the neutralized filtrates with *L. arabinosus* (Wright and Skeggs, 1944). The same filtrates were assayed for pyridoxine with *Saccharomyces cerevisiae* (Atkin *et al.*, 1943). Moisture content was determined by drying 2-g samples at 130°C for 1.5 hours in a forced-draft oven.

Results

The results of the analyses of grain sorghums are contained in Table I. Waxy and nonwaxy varieties show essentially the same variations. Thus there seems to be no correlation between vitamin content and the type of starch stored in the grain. The riboflavin content is only slightly higher than that of yellow hybrid corn. Assays ranged from 0.81 to 2.03 μg per gram, with an average of about 1.3 μg. On the other hand, grain sorghums are 2.5- to 3-fold richer than corn in biotin. Two samples, a waxy kafir (T.S. 25289) from Lubbock, Texas, and Midland from Hays, Kansas, in 1944 were low in comparison with other sorghums, yet contained quantities of biotin equal to

TABLE I

VITAMIN CONTENT OF GRAIN SORGHUMS

Variety	Crop year	Place grown	Vitamin content—moisture-free basis				
			Riboflavin	Nicotinic acid	Pantothenic acid	Biotin	Pyridoxine
			μg/g	μg/g	μg/g	μg/g	μg/g
Waxy sorghums							
Sagrain	1939	Stoneville, Miss.	0.95	27.7	4.6	0.28	2.8
Leoti	1941	Lincoln, Nebr.	.81	37.6	7.4	.23	3.5
Leoti	1942	Hays, Kansas	1.31	34.1	9.9	.28	4.0
Cody	1942	Hays, Kansas	.96	72.1	9.8	.24	5.1
Cody	1943	Hays, Kansas	.96	72.9	7.6	.24	4.8
Leoti×Atlas, H.C. 40–17	1943	Hays, Kansas	1.41	50.2	8.5	.30	7.3
Leoti×Atlas, H.C. 41–13	1943	Hays, Kansas	—	54.0	8.9	.28	8.6
Waxy kafir, T.S. 25289	1943	Lubbock, Texas	1.32	53.1	9.7	.09	7.9
Waxy milo, S.A. 5404–1	1943	Lubbock, Texas	1.56	55.9	8.1	.21	6.6
Waxy Leoti×71–2–13–9	1943	Woodward, Okla.	.84	43.8	9.9	.11	5.1
Texas Waxy kafir	1943	Lincoln, Nebr.	1.26	58.7	10.3	.28	7.9
Cody	1944	Hays, Kansas	1.43	70.5	8.7	.24	6.8
Leoti×Atlas, H.C. 42–27	1944	Hays, Kansas	.96	48.4	9.2	.27	6.8
Cody×Wonder Club, H.C. 44–2045	1945	Hays, Kansas	1.41	83.1	8.6	.32	7.1
Cody×Wonder Club, H.C. 45–112	1945	Hays, Kansas	1.59	91.9	8.9	.30	4.8
Cody×Wonder Club, H.C. 45–140	1945	Hays, Kansas	1.58	83.2	7.8	.34	4.6
Yellow Waxy hybrid, H.C. 45–5173	1945	Hays, Kansas	1.50	78.8	7.9	.36	4.7
Yellow Waxy hybrid, H.C. 45–5177	1945	Hays, Kansas	1.68	71.4	7.4	.33	4.7
Cody	1945	Hays, Kansas	1.29	71.8	8.7	.21	4.2
Waxy Atlas, H.C. 41–20	1945	Hays, Kansas	1.39	45.5	7.8	.35	5.2
Leoti×Atlas, H.C. 42–29	1945	Hays, Kansas	.90	50.5	10.2	.30	5.3
Leoti	1945	Hays, Kansas	1.74	36.3	12.0	.46	6.3

TABLE I—*Continued*

Variety	Crop year	Place grown	Vitamin content—moisture-free basis				
			Riboflavin	Nicotinic acid	Pantothenic acid	Biotin	Pyridoxine
			μg/g	μg/g	μg/g	μg/g	μg/g
Nonwaxy sorghums							
Atlas	1942	Hays, Kansas	1.11	35.7	5.9	0.23	4.8
Wonder Club	1942	Hays, Kansas	.65	65.2	6.9	.22	5.2
Kalo	1943	Hays, Kansas	1.59	43.4	5.6	.28	6.0
Early Kalo	1943	Hays, Kansas	1.06	31.4	5.6	.29	5.4
Wonder Club	1943	Hays, Kansas	1.22	70.0	6.1	.18	8.4
Texas Blackhull kafir, T.S. 9195	1943	Lubbock, Texas	1.11	33.7	5.2	.32	7.3
Texas milo, T.S. 25243	1943	Lubbock, Texas	2.03	66.2	8.7	—	—
Club kafir	1944	Woodward, Okla.	.90	56.7	6.3	.25	8.0
Midland	1944	Hays, Kansas	1.69	45.9	5.8	.09	7.0
Club kafir	1945	Hays, Kansas	1.11	45.4	7.5	.36	6.4
Club×Day, H.C. 40–431	1945	Hays, Kansas	.93	54.7	10.6	.39	6.5
(Leoti×Club)×Custer, H.C. 44–115	1945	Hays, Kansas	1.32	38.3	6.1	.38	6.9
Midland×Wonder Club, H.C. 44–2112	1945	Hays, Kansas	1.24	57.3	5.8	.35	6.2
Midland×Wonder Club, H.C. 44–2117	1945	Hays, Kansas	1.54	72.6	7.6	.40	7.5
Cody×Wonder Club, H.C. 45–110	1945	Hays, Kansas	1.53	76.6	6.2	.37	4.7
Martin	1945	Hays, Kansas	1.46	34.3	10.3	.36	6.5
Westland	1945	Hays, Kansas	1.75	49.1	11.7	.39	7.5
Midland	1945	Hays, Kansas	1.32	48.9	4.6	.29	5.3
White durra	1945	Davis, Calif.	.82	53.9	9.8	.34	2.1
Double Dwarf Yellow milo	1945	Davis, Calif.	1.00	73.9	14.8	.38	2.5

that found in corn. While variation is noted in the pyridoxine levels, most samples contained only slightly less than corn.

Pantothenic acid levels ranged from 4.6 to 14.8 μg per gram. Analyzing other varieties, Knox *et al.* (1944) reported values of approximately 12 to 15 μg per gram.

Grain sorghums are notably richer than corn in nicotinic acid. Furthermore wide variation exists in the several varieties reported. Values range from 27.7 μg per gram for Sagrain to 91.9 μg per gram for a Cody × Wonder Club selection (H.C. 45–112). Similar differences were reported by Knox *et al.* (1944).

Discussion

The wide differences found in nicotinic acid levels suggested that by planned hybridization the content of this vitamin may be increased over that found in present commercial varieties. Several examples support this hypothesis. The Cody variety, now of particular interest because of its waxy starch, represents a selection from a Leoti × Club cross. The three Leoti samples which were analyzed contained 37.6, 34.1, and 36.3 μg per gram of nicotinic acid, while two Club Kafir samples contained 56.7 and 45.4 μg per gram. Yet Cody of the 1942–1945 crop years contained 70.5 to 72.1 μg per gram. Further evidence supporting this view is contained in the data for the Leoti × Atlas crosses. The Atlas sample contained 35.7 μg per gram, approximately the same level as found in Leoti. Strains of the cross that were tested contained 48.4 to 54.0 μg of nicotinic acid per gram. Two yellow waxy hybrids of unknown parentage (H.C. 54–5173 and H.C. 45–5177) are of interest also, since they contained 78.8 and 71.4 μg per gram of this vitamin.

A nonwaxy hybrid, Wonder Club, contained 65.2 μg of nicotinic acid per gram. This variety is thought to be a cross of kafir × feterita. Knox *et al.* (1944) reported a series of standard kafir strains to contain 28.8 to 33.6 μg per gram (different crop years and locations). Somewhat higher values are reported in this paper for kafir types, ranging from 33.7 to 56.7 μg per gram. Feterita strains are reported to contain nicotinic acid in the range of 43.7 to 56.2 μg per gram (Knox *et al.*, 1944).

Furthermore, crosses involving varieties such as Cody and Wonder Club, both of which contain unusually large amounts of nicotinic acid, resulted in progeny of which some contained even more nicotinic acid. Three waxy selections are reported, containing 83.1 to 91.1 μg of nicotinic acid per gram, while a nonwaxy selection contained 76.6 μg. These differences may be attributable to chance selection.

Though some variation occurs in the levels of the other vitamins, and though wide differences between maximum and minimum values for pantothenic acid and pyridoxine contents are reported, the evidence does not suggest that the contents of B-complex vitamins other than nicotinic acid can be altered appreciably by hybridization. The pyridoxine contents of the two California-grown varieties, White durra and Double Dwarf Yellow milo, as well as that of the Sagrain from Mississippi, are substantially lower than those found in other varieties from elsewhere. Cody sorghum grown at Hays, Kansas, varied in content from year to year as follows: 1942, 5.1 μg per gram; 1943, 4.8; 1944, 6.8; 1945, 4.2. In general, variations in riboflavin contents were not appreciable, though seven samples contained less than 1.0 μg per gram. Such variation in vitamin levels may be the result of variations in soil fertility, climatic conditions, and other factors.

Summary

Analyses are presented for the riboflavin, nicotinic acid, pantothenic acid, biotin, and pyridoxine contents of 42 samples of grain sorghums. Compared to corn, they contain approximately the same quantities of riboflavin and pyridoxine and more pantothenic acid, nicotinic acid, and biotin.

The data suggest that nicotinic acid contents may be significantly increased through hybridization. The Cody variety (70 to 72 μg per gram) represents a cross of Leoti (approximately 35 μg per gram) and Club (approximately 45 μg per gram). Further increases, to as high as 91.9 μg per gram, were found in Cody × Wonder Club strains.

Acknowledgment

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IS THE DEGREE OF GRITTINESS OF WHEAT FLOUR MAINLY A VARIETAL CHARACTER?

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A property of wheat flour that is of great practical importance is its structure or grittiness, i.e., its greater or lesser tendency to form into lumps. Flour that forms lumps is more difficult to use than flour that pours almost as smoothly as fine dry sand. The nonlumping type is easy to work up into a homogeneous mixture, whether for baking, sauces, or thickenings.

It is no doubt rather generally assumed that grittiness depends on protein content. Some support for this opinion is found in the fact that Manitoba spring wheat of high protein content mills into a flour possessing great grittiness, whereas the improved winter varieties of northern Europe with their generally low protein content yield a flour of unsatisfactory grittiness. As will be shown below, there is no valid evidence for the accuracy of this assumption.

In the wheat-breeding work at Weibullsholm attention has been directed to possible genetic factors, independent of the crude-protein content, that might affect the grittiness in flour. The first observations pointing in that direction were made some 10 years ago when a number of new winter wheat crosses with the Hungarian variety Bánkuter 178 as a parent were test milled for baking studies. Among these new varieties it was astonishing to find some which, while remarkably easy to mill, gave a flour that possessed a high degree of grittiness, deviating widely from that of the common Swedish winter varieties. In the course of years a number of such varieties were found and examined, but the majority were rejected because of unsatisfactory agronomic qualities. One variety possessing grittiness,

however, was made available to agriculturalists in 1943. It is Weibulls' Eroica wheat, which was raised from the cross (Bánkuter 178 × Standard) × Äring. Thanks to its combination of such valuable characters as relatively good winter-hardiness, very stiff straw and—compared with its rivals—very superior grain-yield, it has now attained a dominating distribution in the plains of southern Sweden. Under identical nitrogen-manuring conditions, Eroica wheat has a somewhat lower protein content than other Swedish winter varieties of wheat. Accordingly, it is remarkable that Eroica should deviate so much from its competitors in respect to grittiness. That this variety belongs to a distinct class is immediately evident from visual inspection of poured-out flour samples, and this has been fully confirmed in the sifting tests reported in this paper.

Methods

The material was milled on a Buhler laboratory mill having a 0–60 % extraction capacity. For every test 100 g of flour were used, and sifting continued for 30 minutes. A shaker type of sifter was used, driven at a speed of about 340 strokes per minute. The recorded values are the means of three different tests, thus allowing of a statistical treatment of the material.

Results and Discussion

Winter Wheat Trials. Table I shows the results of yield trials for a series of winter wheat varieties grown under uniform conditions. The

TABLE I

RELATION BETWEEN WHEAT VARIETY, PROTEIN CONTENT, AND GRITTINESS OF FLOUR
(Winter Wheat Variety Trials, 1945)

Variety	Protein (d.m. basis)	Grittiness		
		Above		Through cloth 15
		Cloth 12	Cloth 15	
	%	%	%	%
Standard	8.6	2.8	16.2	81.0
Standard II	8.9	4.6	15.0	80.4
Äring II	9.4	3.4	15.2	81.4
Äring III	9.0	3.7	15.2	81.1
Eroica	8.4	7.0	27.8	65.2
Skandia II	8.9	3.8	16.2	80.0
Skandia III	8.6	4.0	15.5	80.5
Borg	9.1	4.2	17.1	78.7
Standard error [1]		0.44	1.52	1.92

[1] Applies to mean values for three determinations.

protein content of the flour was lowest in the Eroica wheat, 8.4%, and highest in Äring wheat II, 9.4%. Grittiness data show that in spite of having the lowest protein content Eroica wheat occupies an absolutely distinct position; only 65.2% of the flour passes through cloth 15, while the corresponding percentages for the other varieties lie between 78.7 and 81.4%. The difference between the last two values is not statistically significant. On the other hand, the considerably divergent figure for Eroica wheat must be regarded as a significant expression of the exceptional position occupied by that variety.

TABLE II

RELATION BETWEEN WHEAT VARIETY, PROTEIN CONTENT, AND GRITTINESS OF FLOUR
(Winter Wheat Variety Trials, 1945)

Variety	Protein (d.m. basis)	Grittiness		
		Above		Through cloth 15
		Cloth 12	Cloth 15	
	%	%	%	%
New variety out of Eroica × Virtus	8.8	4.7	19.2	76.1
Virtus	8.0	3.8	13.3	82.9
Ergo	8.1	4.3	17.7	78.0
Eroica	7.7	6.8	27.2	66.0
New variety from Eroica × Virtus	7.8	8.6	26.0	65.4
New variety from Eroica × Virtus	8.3	5.9	25.8	68.3
Standard error [1]		0.73	2.28	2.93

[1] Applies to mean values for three determinations.

A second trial with winter wheat was made with Ergo, Eroica, and Virtus, and with three new varieties obtained from the cross between Eroica and Virtus. Eroica has a high grittiness, while Virtus is not satisfactory in this respect. The results of this second trial are given in Table II. Eroica again shows the lowest protein content, 7.7%, while the highest figure is 8.8%. In grittiness, two of the varieties raised from the cross Eroica × Virtus are fully equal to the mother variety Eroica. The third is somewhat better than the paternal variety Virtus, but both these varieties and Ergo must be assigned to the class possessing unsatisfactory grittiness.

The data given in Tables I and II show no connection whatever between crude protein content and degree of grittiness. The high grittiness that is characteristic of Eroica wheat as well as of two of the progenies of Eroica × Virtus must be due to other hereditary factors,

which have apparently admitted of being rather freely recombined at crosses.

Spring Wheat Trials. Results from a variety trial of spring wheats are presented in Table III. By comparison with the winter varieties (Tables I and II), all spring varieties, except Fylgia, show rather high to very high grittiness. Fylgia wheat seems to belong to the unsatisfactory class, as 81.7% of the flour passed through cloth 15. Corresponding values for other varieties ranged from 61.1 to 69.2%. A statistically significant difference exists between the value for Fylgia and those for other varieties, and there are also some other significant differences. The decidedly divergent type of grittiness possessed by Fylgia wheat would thus seem immediately clear.

TABLE III

RELATION BETWEEN WHEAT VARIETY, PROTEIN CONTENT, AND GRITTINESS OF FLOUR

(Spring Wheat Variety Trials, 1945)

Variety	Protein (d.m. basis)	Grittiness		
		Above		Through cloth 15
		Cloth 12	Cloth 15	
	%	%	%	%
Atle	10.1	5.5	26.8	67.7
Progress	9.7	7.2	31.7	61.1
Brons	10.6	6.3	28.3	65.4
Kärn	10.5	5.6	25.2	69.2
Diamant II	10.5	9.0	24.6	66.4
Fylgia	10.0	3.8	14.5	81.7
New variety from 8388 × 8473	9.4	6.3	26.8	66.9
New variety from 4099 × C 1833	8.9	6.6	26.7	66.7
Standard error [1]		0.99	1.76	2.12

[1] Applies to mean values for three determinations.

Comparison of the grittiness of the different varieties with their respective protein contents again provides no support for the assumption that the property of grittiness depends on the protein content. A notable fact is that the two good new varieties listed in Table III gave much higher grain yields than the market varieties, and accordingly show a relatively low crude-protein content, but are nevertheless relatively gritty. Another interesting point is that Brons wheat and Fylgia wheat—raised from the crosses Aurore × Extra Kolben, respectively, and therefore very closely related—are very different in grittiness though differing in protein content by only 0.6%. Possibly Fylgia inherited its unsatisfactory grittiness from its mother Aurore,

while the high degree possessed by Brons was transmitted from the father variety Extra Kolben II.

Manuring Trials. The dominant part that the variety, rather than the protein content, plays in grittiness is also illustrated by the results of nitrogen-manuring trials at Weibullsholm. The same wheat variety was supplied with different quantities of nitrogenous fertilizer —some applied as late as during the period of earing—and considerable differences in protein content were thus obtained. Had there been any correlation between protein content and grittiness, this would have manifested itself in the results from these nitrogen-manuring trials.

TABLE IV

EFFECT OF NITROGEN FERTILIZATION ON PROTEIN CONTENT AND GRITTINESS

(Winter Wheat Variety Standard II, 1945)

Calcium nitrate added kg/hectare			Protein (d.m. basis)	Grittiness		
Date of application				Above		Through cloth 15
3/5	22/6	26/6		Cloth 12	Cloth 15	
			%	%	%	%
			7.3	4.0	15.5	80.5
200			7.7	4.0	17.0	79.0
350			8.6	4.3	16.2	79.5
200	150		8.9	4.5	18.3	77.2
500			9.1	4.2	17.0	78.8
200	300		9.7	4.1	18.0	77.9
800			11.0	4.5	16.4	79.1
200	300	300	11.8	3.5	14.5	82.0
Standard error [1]				0.12	0.44	0.52

[1] Applies to mean values for three determinations.

The results of tests undertaken on materials from a nitrogen-manuring trial of this kind with the winter wheat variety Standard II and with the spring variety Diamant II are recorded in Tables IV and V. The former shows protein contents rising evenly from 7.3 to 11.8%. The data for the degree of grittiness show only minor differences (though some are statistically significant) between different sections of the trial, but no correlation with the crude-protein content is discernible. Indeed, the least satisfactory figures for grittiness were obtained in the section with the highest crude-protein content. Table V, like Table IV, offers no evidence of any correlation between grittiness and crude-protein content in Diamant II. It is true that there are statistically significant differences, but these are not attributable to the difference in protein content but probably to the time at which

the nitrogenous fertilizer was applied. A somewhat surprising feature is that the trial-sections with respectively the lowest and next lowest crude-protein contents show the best grittiness figures.

The results of these two fertilizer trials, together with those previously cited from investigations on materials of different varieties, have given a clear verdict that it is primarily the variety and not the crude-protein content which decides the degree of grittiness possessed by wheat flour. Whether other cultivation factors, such as soil and type of year, exercise any influence must here be passed over, there being as yet no material available for the elucidation of this point.

TABLE V

EFFECT OF NITROGEN FERTILIZATION ON PROTEIN CONTENT AND GRITTINESS

(Spring Wheat Variety Diamant II, 1945)

Calcium cyanide applied before showing	Calcium nitrate			Protein (d.m. basis)	Grittiness		
					Above		Through cloth 15
	3/5	19/6	25/6		Cloth 12	Cloth 15	
kg/hectare	kg/hectare	kg/hectare	kg/hectare	%	%	%	%
100				8.9	6.5	28.9	64.6
100	100			8.5	6.1	29.3	64.6
100	50	50		9.4	5.3	24.7	70.0
100	400			9.8	5.0	26.4	68.6
100	100	300		11.9	5.6	29.3	65.1
100	700			12.6	5.1	25.6	69.3
100	100	300	300	15.5	6.5	26.0	67.5
Standard error [1]					0.24	0.73	0.83

[1] Applies to mean values for three determinations.

Relation between Grittiness and Vitreous Kernels. According to the present writer's experience, it appears that wheat varieties that combine a low protein content with a high degree of grittiness contain a higher percentage of vitreous or semivitreous kernels than varieties with an equally high protein content and an unsatisfactory degree of grittiness. Thus, the vitreousness is not considered to depend solely on the protein content, an assumption that is confirmed by investigations made by Mr. Widebäck.[1]

Table VI presents the results of a study of the vitreousness and the protein content of two varieties with an unsatisfactory grittiness, Virtus and Standard, and of two varieties of satisfactory grittiness, Eroica and a new variety designated W:s 11376. The last-mentioned

[1] Mr. I. Widebäck has made quality tests for the Weibullsholm Institute for many years, and his permission to present the following results is gratefully acknowledged.

variety has been shown by sifting tests to belong to the same good class of grittiness as Eroica wheat. The materials for these tests were also grown under comparable conditions of soil, manuring, and so on.

The data show that Eroica and 11376, with their high degree of grittiness, have a decidedly greater tendency to vitreousness than the varieties Virtus and Standard. This is especially remarkable in the case of Eroica wheat, which had an average crude-protein content of only 7.8%, as against 8.2–8.3% in the other varieties. Hence the figures confirm the fact that vitreosity can to some extent depend on

TABLE VI

RELATIONSHIP BETWEEN VITREOUSNESS, CRUDE-PROTEIN CONTENT, AND SIZE OF THE STARCH GRANULES

Variable	Virtus		Standard		Eroica		W : s 11376	
	Kernels	Protein	Kernels	Protein	Kernels	Protein	Kernels	Protein
	%	%	%	%	%	%	%	%
Kernel								
Vitreous	4.8	11.0	3.2	10.8	8.0	10.4	12.6	10.3
Semivitreous	19.0	8.5	16.0	8.9	26.0	8.1	22.2	8.6
Floury	76.2	7.9	80.8	7.9	66.0	7.3	65.2	7.7
Mean protein content		8.2		8.2		7.8		8.3
Starch granules, % smaller than 0.02 mm		64.0		54.0		71.0		69.0
Grittiness	Unsatisfactory		Unsatisfactory		Very satisfactory		Very satisfactory	

other factors than the crude-protein content, factors linked up with the genetically controlled properties of the variety. If this specific vitreousness, which does not depend on the vitreousness caused by an increasing protein content, exercises an influence upon the grittiness, the next question will be from what source the former kind of vitreousness originates.

The problem of the vitreosity has already been made the object of tests by several investigators. In the report of his results Mr. Widebäck refers to the following authors: Neumann (1929), Walldén (1923), and Hoffmann-Mohs (1931). Müller (1942) presents a short account of the vitreousness and its causes, a translation of which runs as follows: "On being soaked and sectioned the air-dried barley and wheat grains called 'melede' (floury) exhibit a snow-white, flutelike endosperm that is porous and friable. The grains are called 'glassede' (vitreous) when the endosperm is hard, firm, transparent and exhibits a more or less dark section-surface. Between these types there are regular transitions. The more air vacuoles the more floury is the endosperm; in the vitreous endosperm there are no air vacuoles. The vitreous

endosperm contains in solitary parts of the grain somewhat larger amounts of small starch granules than the floury type."

The four varieties mentioned above were analyzed as to their content of starch granules of smaller size than 0.02 mm by Mr. Widebäck. The results of these tests are given in the last column of Table VI. Eroica and W:s 11376 contained respectively 71 and 69% of starch granules smaller than 0.02 mm, as against 64 and 54% for Virtus and Standard. Even though investigations on a larger series are desirable to establish more reliable means, the results of the present analyses provide an acceptable explanation of the higher vitreousness in the varieties Eroica and W:s 11376 as compared with Virtus and Standard. The cause may well be simply that the comparatively large amounts of small starch granules contained in the two former varieties, together with the protein substance present, constitute a more compact endosperm and in that way prevent refractive air interspaces from being formed; while in the two latter varieties, with their larger starch granules, the mass is less compact and consequently more likely to contain air vacuoles, which makes the kernel more floury.

There is a possibility that the degree of grittiness is correlated with the vitreousness thought to be specifically linked to the variety and to owe its origin to the size of the starch granules. To settle the matter definitely more thorough investigations are required than those now undertaken. Other explanations of the differences in the grittiness of the varieties are conceivable; e.g., differences in size of the protein molecules or differences in the chemical composition of the proteins.

It may be mentioned that tests of the gluten quality were made by the Berliner-Koopmann method and showed that Eroica wheat has a higher swelling index than other South-Swedish varieties. Still, the high degree of grittiness possessed by the Eroica wheat cannot be ascribed to this fact, for a simultaneous test of Virtus wheat gave a still higher swelling index in spite of the unsatisfactory grittiness possessed by this variety.

It should be pointed out that Eroica wheat, as distinct from other winter varieties of Swedish wheat, is fairly closely allied to certain spring varieties. Bánkuter 178, the maternal grandmother of the Eroica wheat, is in all probability the result of a spontaneous cross between a Hungarian winter wheat and the spring variety Marquis. Marquis in its turn is an offspring of the Hard Red Calcutta × Red Fife. All the Swedish spring varieties originate from Svalöf's Kolben wheat, a variety that according to Åkerman (1934) is very closely related to Red Fife. In view of the relationship between the species there is a possibility it may be the same or essentially the same hereditary factors that, independent of the protein content, are responsible

for the relatively high degree of grittiness in the majority of our spring wheat varieties as well as in Eroica wheat and in some new winter varieties now undergoing tests.

For future wheat-breeding work it is naturally of great interest that the property of grittiness has proved to be so strongly linked to the variety and is not dependent on the protein level. It will mean that this property can be freely recombined by means of systematic crossings and selection of progenies. In view of the present state of the problem of grittiness and the importance this property has for the quality of the flour, it may finally be pointed out that in future wheat-breeding work in northern Europe this property ought to receive greater attention than has hitherto been awarded it. The aim must be to ensure that new wheat varieties shall possess, in addition to other satisfactory properties, a degree of grittiness that will meet legitimate requirements.

Summary

The milling industry requires a flour that is gritty and shows little tendency to agglomerate, and it has been generally thought that a correlation exists between grittiness and protein content.

Varieties such as Eroica have now been produced that combine high grittiness with low protein content. Investigations in which grittiness was measured by sifting tests on flours milled from both winter and spring wheats show clearly that it is variety and not the protein content that controls grittiness. Confirmatory data were obtained in trials in which protein content was varied by different levels of nitrogenous fertilizers, and no correlation between protein content and grittiness was found.

It has been observed that varieties that are gritty possess greater vitreousness of kernels in relation to protein content. Virtus and Standard with 23.8 and 19.2% vitreous and semivitreous kernels have unsatisfactory grittiness, whereas Eroica and W:s 11376 with 34.0 and 34.8% vitreousness have satisfactory grittiness. The explanation of greater vitreousness is thought to be the large amount of small starch granules in the latter two varieties, and it is possible that grittiness is correlated with this property. No correlation exists between gluten swelling index and grittiness.

Pedigrees of the varieties are discussed and it is pointed out that the hereditary factors in Swedish wheats that control grittiness may come from Red Fife, which is one of the original parents of both the winter wheat Eroica and the spring wheat Kolben.

Acknowledgment

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EXTRANEOUS MATERIALS IN GROUND SPICES AND CHOCOLATE PRODUCTS [1]

NILES H. WALKER [2] and GASTON DALBY [3]

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The 1945–46 Committee of the New York Section, in addition to its study of procedures for the determination of extraneous matter in flour (Walker, 1947), made a partial survey of methods applicable to other ingredients used in bakery products as well as the general condition of such samples in regard to extraneous matter. Among these, spices were found to be a serious possible source of contamination. Blumberg (1939) studied the infestation of various drugs, including many spices, with *Tribolium*. He was interested in the ability of *Tribolium* beetles to live and reproduce in the spices rather than in determining any extraneous matter that might be present. Greenish and Braithwaite (1910) published a method for the identification of insect fragments in spices which consisted of the treatment of a suspension of the spice in water with an oxidizing mixture of sulfuric and chromic acids for periods of 36–48 hours. Such a procedure would preclude the counting of rodent hairs and softer parts of insects since these would be destroyed by the reagents used. Other than the work of Blumberg, Harris (1946) in a recent bibliography of papers on sanitation lists nothing on the subject of spices and chocolate products.

The committee had in mind the following general objectives in its plan for the development of a procedure applicable to ground spices and chocolate. (Chocolate is classed with spices because of the rather general similarity in physical nature.)

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[1] Report of the 1945–46 and the 1946–47 Committees of the New York Section on Procedures for the Examination of Food Products for Extraneous Materials.

[2] Chairman 1945–46 Committee. National Biscuit Company, New York, N. Y.
[3] Chairman 1946–47 Committee. Ward Baking Company, New York, N. Y.

1. Application of the flour procedure developed by the committee to spices without too great modification.
2. Elimination of, if possible, the necessity of removing the oily material from the spices as a preliminary step.
3. Digestion and extraction of extraneous matter under acid conditions.

A procedure for each type of food product is a source of confusion to the analyst, and the committee considered it desirable to adapt previously recommended procedures to spices with as little modification as practicable. The removal of the oils and fats from spices and chocolate products before the separation procedure is begun is time consuming; also extraneous matter may be lost at this stage unless great care is taken. Work of the Federal Security Agency (U. S. Food and Drug Administration, 1944) as well as that of the Committee indicates in general that extraneous matter is more easily and completely extracted under acid conditions than neutral or alkaline.

The recommended A.O.A.C. procedures (1945) for spices involve benzine extraction of the oil from the spice, suspension of the dry fat-free spice in water, followed by gasoline extraction of the extraneous matter using the Wildman trap technique. In the case of the *Capsicums*, the dry fat-free spice is digested in a borax solution before extraction with gasoline.

In the A.O.A.C. procedures it is necessary in many cases to work with fat-free spice because the oily spice particle is of low specific gravity and also resists being wetted by the water phase; both conditions tend to carry the spice particle over into the gasoline layer during separation of the extraneous matter. It might be said, therefore, that an oil phase is removed as a preliminary step, then an oil phase (gasoline) is added during the separation of the extraneous matter.

Experimental

In view of these facts the committee considered the possibility of adding a solvent to the spice which would either dissolve or disperse the oil phase and permit the spice particle to be wetted by the water phase when it was added; the oil of the spice not to be removed during the procedure. Many solvents were tried, but acetone proved to have the best characteristics. In addition to its solvent properties, acetone apparently tends to loosen extraneous matter, especially rodent hairs, from the spice particles. An explanation of this action may lie in the solvent and softening effect of acetone on the resins and resinous-like material present in many spices.

Preliminary trials indicated that the acetone procedure was hopeful. Collaborative samples of Saigon cinnamon were distributed among the committee members. The objectives were to test the method in general, and specifically to find the best proportion of acetone to water and acid used. Concentrations of 40% acetone and higher had failed to show improvement in the condition of the field over lower concentrations. The boiling point of the mixture with the higher concentrations of acetone also tended to be too low for rapid digestion of materials present in many spices.

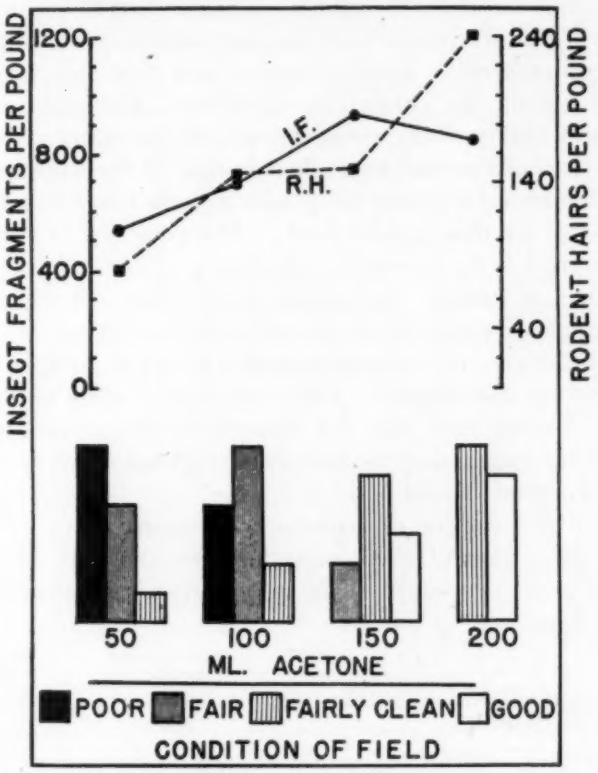

Fig. 1. Average collaborative results obtained with various quantities of acetone compared with the condition of the field as reported by the collaborators.

Ten grams of Saigon cinnamon were added to 50, 100, 150, and 200 ml of acetone in a liter Florence flask. To this were added 380 ml of water, 20 ml of concentrated hydrochloric acid, and 20 ml of light mineral oil and the mixture refluxed for about 20 minutes. The mineral oil layer was separated, washed, filtered through a lined paper, and examined in the usual manner as described by Walker (1947). The average collaborative results are shown in Figure 1. The extraneous matter counts and the condition of the fields show that 200

ml of acetone is the best quantity to use under the conditions of the test.

Six collaborators determined extraneous matter in the same sample by the A.O.A.C. procedure. The comparative results approximated to a one-pound basis are as follows:

	Insect fragments per lb.	*Rodent hairs* per lb.
A.O.A.C. procedure	429	23
Acetone procedure (200 ml)	844	240

The tentative acetone method was then revised in accordance with the results of this study and with the suggestions of the collaborators. A rather comprehensive group of spices and chocolate products was distributed among the committee members. The objective was to test the applicability of the revised procedure to many types of products. No one collaborator was asked to test all the samples.

The committee feels that the results reported in Table I are satisfactory checks for this type of work. The objective of a test for extraneous matter is to determine whether a material is clean, lightly contaminated, or heavily contaminated. Evidence is increasing that uniform samples are not obtainable even after the most thorough mixing. The adherence of extraneous matter to spice particles could well prevent uniform distribution. Only one type of spice studied by the committee, ground sage, did not respond to the acetone procedure. The light leafy material of the sage came through into the mineral oil layer and obscured the field.

The identical sample of paprika was examined by the 1945–46 committee using the A.O.A.C. procedure and the 1946–47 committee using the acetone procedure. The average counts approximated to a one-pound basis were as follows:

	Insect fragments per lb.	*Rodent hairs* per lb.
A.O.A.C. procedure	522	102
Acetone procedure	586	165

Once again the suggestions of the collaborators were incorporated in a final revision of the procedure.

Final Acetone Procedure for Determination of Extraneous Materials in Ground Spices and Chocolate Products

Place a 10-g sample of ground spice in a dry liter Florence or similar type flask. Use a 50-g sample for cocoa and chocolate liquor. Add 200 ml of acetone and swirl flask until solvent and spice are mixed. (In the case of chocolate liquor, the sample and acetone may be refluxed for a few minutes at this stage so as to disintegrate the material.)

TABLE I

RESULTS OF COLLABORATIVE STUDY OF EXTRANEOUS MATERIALS IN A SERIES OF SPICES AND CHOCOLATE PRODUCTS

(Per lb. basis[1])

Coll.	I.F.	R.H.	Coll.	I.F.	R.H	Coll.	I.F.	R.H.
GROUND ALLSPICE			GROUND CARAWAY			GROUND CARDAMON		
H	1232	158	A	630	90	C	270	0
I	270	135	C	1350	90	D	1555	0
J	450	45	I	270	405	E	1271	0
GROUND CAYENNE			CHOCOLATE LIQUOR			GROUND CLOVES		
A	1170	45	D	108	0	C	270	360
C	5580	0	E	72	0	H	450	90
D	630	45	H	18	12	J	182	90
H	5130	0	J	18	0			
I	3510	90						
J	7446	90						
COCOA			GROUND CORIANDER			GROUND GINGER		
A	18	27	B	180	0	A	90	180
F	216	144	C	315	0	E	817	45
H	45	27	D	760	0	I	955	45
J	18	45						
GROUND MACE			GROUND NUTMEG			GROUND PAPRIKA		
C	4950	90	F	270	180	E	817	90
F	2475	225	G	409	57	G	704	90
I	3150	90	H	360	0	H	338	225
			J	363	0	I	630	315
						J	439	106
GROUND BLACK PEPPER			GROUND RED PEPPER			GROUND TURMERIC		
C	225	135	B	182	0	B	90	0
F	90	135	C	315	0	C	90	0
H	825	135	H	495	90	I	135	0
J	295	45	I	900	45			
			J	362	106			

[1] Counts approximated to a one-pound basis.

Add 380 ml of distilled or recently boiled water and 20 ml of concentrated hydrochloric acid. Finally add 20 ml of light mineral oil. Place flask under reflux condenser and boil gently for 20 minutes. Cool to about 25°C and add 50 ml of gasoline or benzine. Decant into separatory funnel. At this stage do not wash Florence flask with

water. After the heavier spice particles have settled, open clamp on separatory funnel and discard the heavy particles. Then draw off water-acetone layer, to within an inch or so of oil layer, into Florence flask and use this solution, which will contain some suspended spice particles, to wash out Florence flask. This procedure may be repeated several times. After the two layers in the separatory funnel have become well separated, draw off water-acetone layer to within an inch or so of oil layer. Thoroughly wash oil layer with water; that is, add water to separatory funnel, draw off water layer to within an inch or so of oil layer, and repeat until water layer is free from spice particles.

Filter oil layer on lined paper, wash separatory funnel and filter paper with isopropyl alcohol or ethyl alcohol, and if necessary wash paper with boiling alcohol. With many spice samples, especially those high in resins, a precipitate of amorphous material may obscure the field. In such a case, turn off suction, flood paper with 25 ml of boiling alcohol, allow to stand a few seconds, and turn on suction. Repeat if necessary. Wet paper with water, and examine under microscope in usual manner. Be sure paper is thoroughly wet during examination. For added details of washing, filtration, microscopic examination, etc., see Walker (1947).

With some spice samples, the quantity of light cellulose material carried over into the oil layer may be so great that the paper cannot be examined under the microscope. In this case wash the spice particles from the paper back into a liter Florence flask with 100 ml of acetone. Add 195 ml of water, 5 ml of concentrated hydrochloric acid, and 20 ml of light mineral oil. Reflux for 20 minutes and repeat washing procedure, filtration, etc. Filter on the same paper that was used the first time. Thirty diameters is recommended as the most convenient magnification. Use 75 diameters to check doubtful particles.

Discussion

The samples used in this study (except the Saigon cinnamon) were chosen at random from commercial samples without any preliminary check on their degree of contamination. The results given in Table II show a serious condition as regards extraneous matter, especially in imported spices. In addition to the collaborative samples, many checks have been made by the committee members on commercial samples by the use of the acetone procedure.

Spices and chocolate are almost entirely imported products. The committee feels that the serious condition in regard to extraneous matter is not a reflection on domestic spice importers and processors. Remedial measures must come from the cooperation of the importers

TABLE II

EXTRANEOUS MATTER FOUND IN SPICES AND CHOCOLATE PRODUCTS USING THE ACETONE PROCEDURE

(Routine examination of commercial samples)

Type of material	Insect fragments per lb.[1]	Rodent hairs per lb.[1]
Allspice, Jamaica	136	0
Allspice, Jamaica	590	136
Allspice, Jamaica	0	0
Allspice, Jamaica	0	0
Allspice, Jamaica	5221	227
Allspice, Jamaica	4041	272
Allspice, Jamaica	1998	0
Allspice, Jamaica	1093	272
Allspice, Jamaica	91	136
Cinnamon, Saigon	136	227
Cinnamon, Saigon	45	0
Cinnamon, Saigon	90	45
Cinnamon, Saigon	0	0
Cinnamon, Saigon	0	0
Cinnamon, Saigon	2500	495
Cinnamon, Saigon	2000	0
Cinnamon, Saigon	3500	180
Cinnamon, Saigon	5000	1125
Cloves, Zanzibar	315	45
Cloves, Zanzibar	45	0
Cloves, Zanzibar	817	908
Cloves, Zanzibar	908	2406
Cloves, Zanzibar	999	999
Cloves, Zanzibar	0	0
Cloves, Madagascar	363	90
Cocoa	1000	27
Cocoa	500	23
Mace, West India	1226	0
Mace, Pedang	2859	363
Mace, No. 1 Siauw	1589	91
Nutmeg, West India	1498	45
Paprika, Spanish	454	136
Paprika, Spanish	136	409
Paprika, Spanish	772	590
Paprika, Spanish	316	726
Paprika, Spanish	454	363
Paprika, California	91	0
Paprika, California	136	0
Red Pepper	363	45
Red Pepper	91	91
Red Pepper	454	91
Red Pepper	772	45
Red Pepper	817	0

[1] Counts approximated to a one-pound basis.

with various government agencies in an attempt to improve sanitary conditions in the handling, storage, and transportation of spices before they reach the United States, as well as improved domestic conditions of storage, handling, and cleaning.

Summary

An acetone procedure for the examination of spices and chocolate products for extraneous matter has been examined collaboratively and found to be applicable. The method gives higher extraneous matter counts than previously available procedures. It is also more rapid and less laborious.

Data on the extraneous matter content of a group of spices and chocolate products are given.

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Acknowledgments

The chairmen wish to express their gratitude to the following committee members who collaborated in this study: R. T. Bohn, General Baking Company, New York, N. Y.; T. R. Fetherston, Griffith Laboratories, Newark, N. J.; O. J. Fiala, Durkee Famous Foods, Elmhurst, L. I., N. Y.; S. M. Jackson, Sunshine Biscuit Company, L. I. C., N. Y.; J. H. Lanning, Continental Baking Company, L. I. C., N. Y.; Wayne Maynard, Fleischmann Laboratories, New York, N. Y.; Grace McGuire, Laboratory of Industrial Hygiene, New York, N. Y.; E. K. Spotts, National Biscuit Company, New York, N. Y.; J. J. Winston, Jacobs Cereal Labs, Inc., New York, N. Y.; and W. H. Ziemke,[4] Quality Bakers of America Corp., New York, N. Y.

The chairmen also wish to express their gratitude to several of the committee members who helped in the preparation and distribution of samples for study.

[4] Present address: Safeway Stores, Inc., San Francisco, California.

THE USE OF THE AMYLOGRAPH FOR FLOUR MALT CONTROL

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C. J. Patterson Company, Kansas City, Missouri

(Presented at the Annual Meeting, May 1946; received for publication January 6, 1947)

Discussions of the function, construction, and operation of the Brabender Amylograph have been published by Anker and Geddes (1944) and by Brown and Harrel (1944). The purpose of the present work is to consider the application of the amylograph to the control of malt flour additions to commercially milled flours and to compare the amylograph method with the methods of control now in use in the milling industry.

The maltose method of controlling these additions measures the end product of a series of enzyme reactions, since the factor determined is the reducing sugar produced by the combined actions of alpha-amylase and beta-amylase.

Within the range of enzyme concentrations customarily encountered in baking technology, the gassing power method of malt control measures the fermentable sugar produced by the same series of enzyme actions.

The amylograph method of control measures primarily the action of alpha-amylase, since the factor determined is the viscosity of the paste obtained by thermal gelatinization of a flour suspension which has been exposed to malt action. It is generally recognized that alpha-amylase is the active enzyme involved in the liquefaction of starch gels. Sandstedt (1938) showed alpha-amylase to be the prime factor in evaluation of malt in flour technology, since beta-amylase is present in excess in flour without malt supplementation.

Experimental

The amylograph, which is essentially a recording viscosimeter, is used to measure the viscosity of a flour-water suspension that has been thermally gelled while the suspension has been and is being submitted to malt activity while undergoing constant temperature increase from room temperature to the point of maximum gel viscosity.

The procedure requires stirring 100 g of flour weighed on a 14% moisture basis, with 470 ml of McIlvaine buffer solution [1] adjusted to a pH of 5.35. This suspension is then treated according to the recommendations of the manufacturer for operation of the machine.

[1] The composition of this buffer is citric acid 0.105% and dibasic sodium phosphate 0.148%.

The amount of flour and buffer is so chosen that flour at optimum amylase activity will give a reading of between 400 and 600 amylograph units. The factor which was measured was the point of greatest consistency, as observation has indicated that this is the most important value obtained with the procedure.

The determination of the optimum concentration of malted barley or wheat flour was based on laboratory bake shop results that were obtained using the sponge-dough technique with a commercial formula. These results were then verified in three commercial bread shops in which various increments of malted barley flour were used with three bread flours of extremely low diastatic activity.

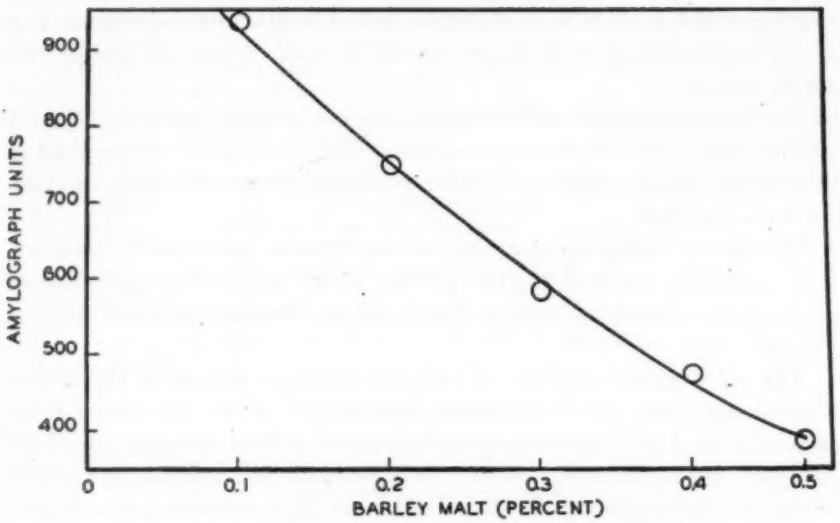

Fig. 1. Relationship between amylograph value and malt concentration.

The increments of malted barley flour were increased from 0.3% to 1.0% in 0.1% steps, and the qualities of the resulting dough and bread were observed. The results from the three shops indicated that the 0.4% increment of malted barley flour produced the best results, with a range of from 0.3% to 0.5% being permissible. Samples of all these flours were then submitted to amylograph analysis by the above procedure, which was designed so that the flours which produced the best results in the shop tests produced an amylograph value of 500, with flours falling within the above mentioned permissible range of amylolytic activity producing amylograph values between 400 and 600 units. This information was then applied to the control of malt flour increments for protracted periods in many commercial bakeries, with results which have in all instances verified the observations made in the original experimentation.

The relationship between amylograph value and the amount of malted barley flour added to wheat flour is presented in Figure 1. All analytical results are reported on a 14% moisture basis.

The amylograph analyses of commercial flours from several mills were compared with the maltose values or gassing power values reported by these mills.

Figure 2 shows the results of comparing the mill maltose value by the Blish-Sandstedt procedure as described in *Cereal Laboratory Methods* (4th edition, page 100), with the amylograph value determined

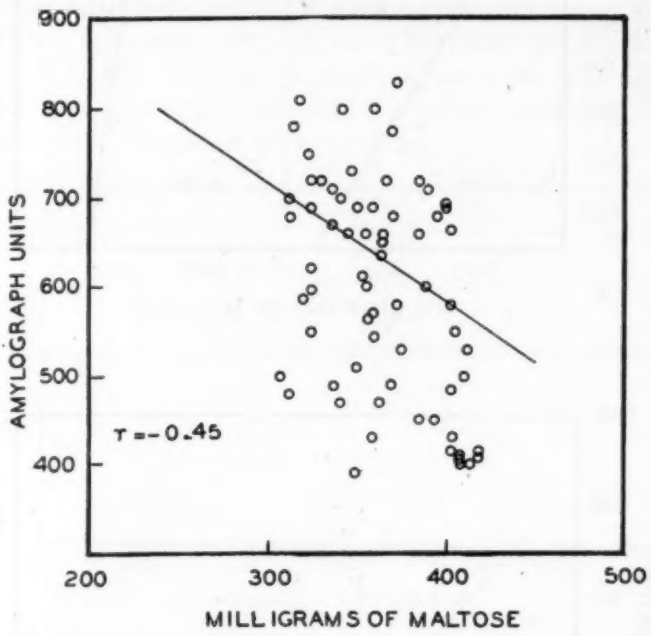

Fig. 2. Comparison of maltose values of flours from several mills with amylograph values.

in this laboratory. Sixty-nine flour samples from several mills are represented. The low significant correlation of −0.45 indicates that little reliance can be placed upon the relationship between the amylograph method and the maltose method as employed by different mills.

Figure 3 is an extension of the data presented in Figure 2, and shows the maltose value plotted against the amylograph value of several samples from four mills. Although significant correlation exists in each case, it is obvious that the relationship of amylograph units to milligrams of maltose varies between mills.

Figure 4 shows the fifth hour gassing power values, determined as described in *Cereal Laboratory Methods* (4th edition, page 101), plotted against amylograph values of 44 flour samples from several mills. The

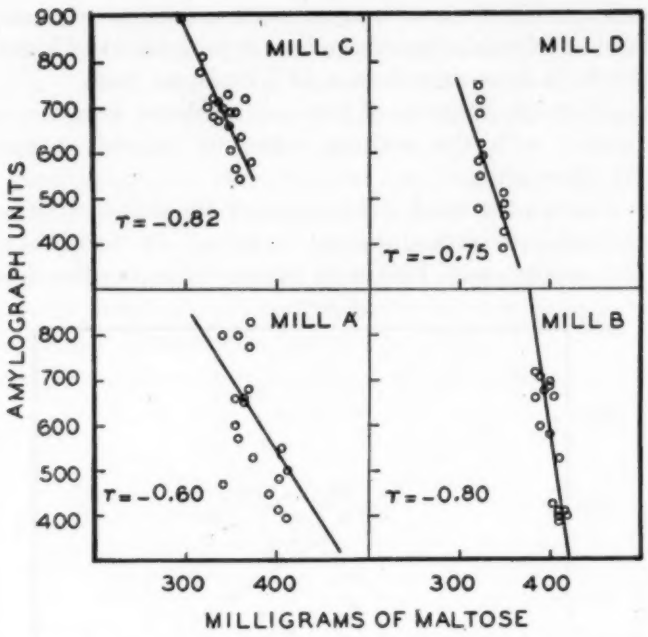

Fig. 3. Comparison of maltose values of flour from individual mills with amylograph values.

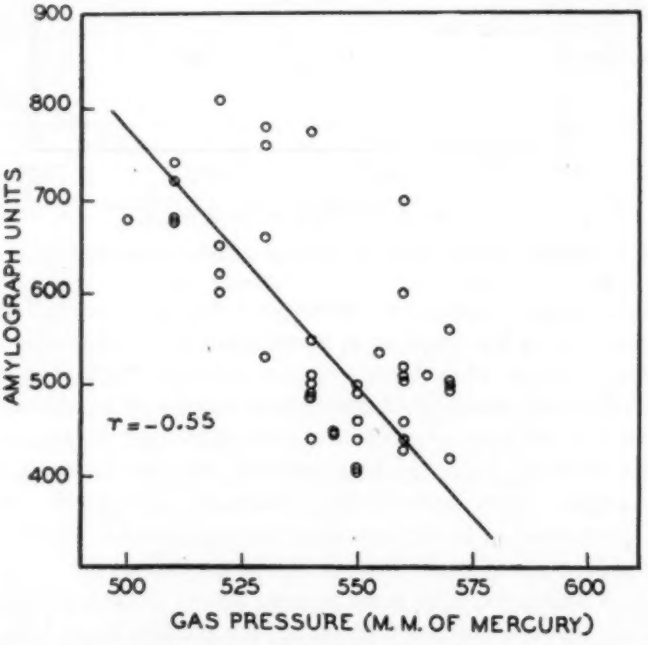

Fig. 4. Comparison of fifth hour gassing power values of flours from several mills with amylograph values.

low significant correlation of −0.55 shown in Figure 4 indicates that a poor relationship exists between the amylograph method and the gassing power method as employed by different mills.

From these results it was obvious that, while in a given flour there may exist a correlation between the conventional methods of evaluation of amylolytic activity and the amylograph method, the nature of this relationship varies between laboratories and between flours to such an extent that in commercial practice the over-all correlation is poor. Therefore, the sensitivity of the various methods to factors known to affect the results of malt evaluation was studied. The effect of storage at various temperatures upon malt values given by the gassing power method and by the amylograph method was investigated and the effects of physical damage produced by grinding upon the values obtained by the three methods were compared.

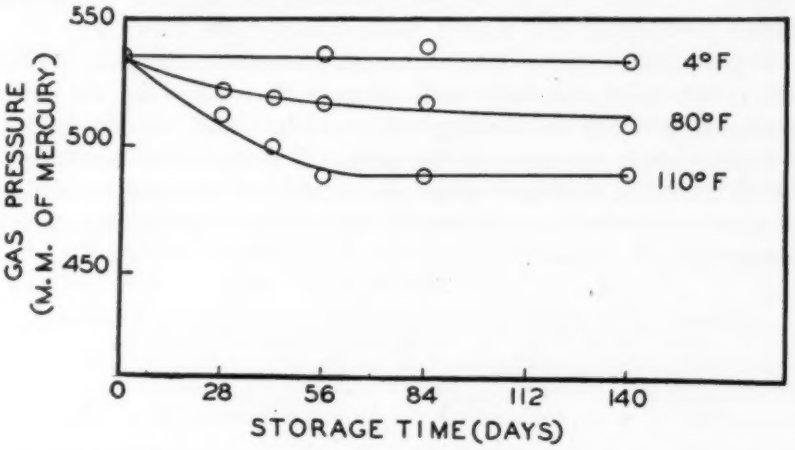

Fig. 5. Effect of storage at various temperatures upon the fifth hour gassing power of flour.

Figure 5 shows the change in gassing power of flour samples that were stored for a period of 140 days in cloth sacks at three different temperatures. Figure 6 shows the change in amylograph readings on the same storage samples.

Table I shows the results of overgrinding upon the amylolytic value of the flour as determined by three methods. The flour was ground with steel balls in a porcelain ball mill.

The amylograph value was affected to some extent by 30 hours of continuous grinding, but the indicated amylolytic value of the flour did not fall outside the optimum baking range.

Seven hours of grinding produced a gassing power sufficient to indicate considerable overtreatment of the flour. Fifteen hours of

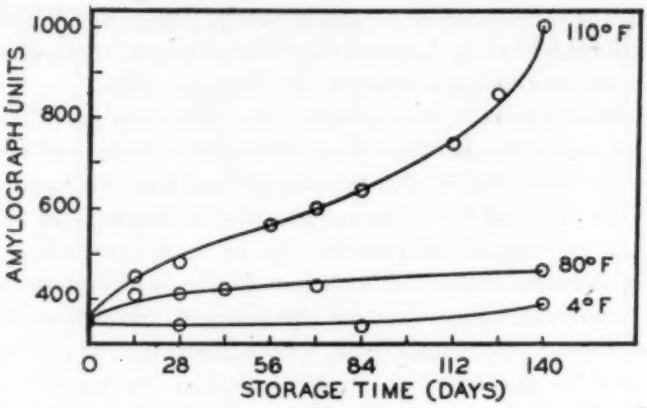

Fig. 6. Effect of storage at various temperatures upon the amylograph value of flour.

grinding increased the maltose value beyond the limits of commercial flours.

Since damaged granules are always produced in commercial milling, these results may give some indication of the reason for the lack of correlation between the amylograph procedure and the conventional methods of malt control used by mills. A slight difference between mills in extent of milling damage can produce an effect upon maltose and gassing power values which will not be shown proportionately by the amylograph method.

TABLE I

EFFECT OF GRINDING UPON THE APPARENT AMYLOLYTIC ACTIVITY OF WHEAT FLOUR AS MEASURED BY VARIOUS METHODS

Grinding period (hours)	Amylograph units	Gas pressure (mm of mercury)	Milligrams of maltose
0	595	553	304
7	560	610	398
15	485	639	436
30	440	642	502
Optimum range	400–600	540–575	370–400

Discussion

The complete role of alpha-amylase in the baking of bread has been studied by a few workers. Kneen and Sandstedt (1946) and Geddes (1946) have discussed the possible functions other than sugar production which this enzyme may have in bread manufacture. Certainly it would seem that if the production of sugar were the primary or most important work of amylase, then a baker should be able to maintain gas production in the dough and produce high quality bread with a total disregard of the amylase activity simply by adjusting the quantity

of sugar in the formula. Such an adjustment has not been found to substitute for amylase activity in producing maximum loaf volume and optimum texture.

As every baker has discovered, gas retention is as important as gas production, and there has been no evidence found in this laboratory to show that gas retention of fermenting dough is affected markedly by amylase. The writers have always associated gas retention with proper conditioning of the flour gluten.

There is strong evidence that places the emphasis on the final effect of the amylases in the oven as being the most important function of malt. Not all of the activity of the amylases takes place in the oven, but the fermentation of the dough piece before entering the oven allows amylolytic activity to condition properly the starch so that when a gel is formed in the oven it will be of the optimum viscosity with the correct level of alpha-amylase activity.

It would seem quite possible that a large portion of the total effect of the amylase on the starch granules actually takes place in the oven during the first stage of baking. The work of Anker and Geddes (1944) indicated that alpha-amylase activity lowers the gelatinization temperature of wheat starch suspensions, as well as decreasing the maximum viscosity reached during gelatinization. If it is assumed that an important function of alpha-amylase is to prepare the starch so that it will gelatinize at the right temperature and to the correct viscosity, then it would be expected that flour deficient in alpha-amylase activity would produce a gel of high viscosity in the oven and that as the exterior of the loaf, which was first exposed to the heat, gelled, the expansion of the loaf would be reduced and the resultant loaf would be small in volume and characterized by a coarse texture.

On the other hand, if the flour is overtreated, then the low viscosity of the starch gel will not establish securely the structure of the loaf and here again there will be some diminution of volume, coalescing of the cellular structure of the crumb, and a dark crumb color.

If the flour has been treated to the optimum range, then the starch viscosity will be low enough to allow for the expansion of the loaf and yet will be high enough so that the established cell structure is of fine and desirable grain.

Although to some extent it is oversimplifying the problem, the writers assume that conditioning of gluten is extremely important for the formation of a finely subdivided dough structure; that, when the temperature reaches 75° to 85°C during baking, the gelling starch assumes the burden which to this point has been carried by the gluten; and that the importance of the gluten as a structural unit is greatly reduced as the starch gel takes over.

Several experiments would appear to justify the conclusion given above. Obermeyer (1940) reconstituted synthetic dough in which gluten was mixed with small particles of glass, round in shape and fractionated to the same size range as wheat starch granules. A synthetic dough was made with yeast, salt, and a small amount of sugar. Except for the coloration of the dough it was normal in appearance during the panary proof and during the very first stages of baking. That is, there was a considerable amount of ovenspring; however, after the initial increase in ovenspring, the entire dough collapsed to the bottom of the pan as a rubbery mass.

Selman (1942) found that, when doughs of very high absorption (100 to 125%) were baked by means of electrical resistance, they all had the typical appearances of normal doughs until a temperature of 76°C was reached, at which point they abruptly became converted into bread although less than 1% of the total water had been evaporated at this stage.

Continued experimentation with doughs of normal absorption ranges indicated that the same phenomenon occurred. However, the change from "dough" characteristics to "bread" characteristics occurred over a much wider range of temperature and was not nearly so sharp as in the doughs containing excess amounts of water.

All of these results indicated that at this stage of bread manufacture the size and texture of the finished loaf are dependent upon the gelatinization characteristics of the starch. Control of this property is, in the authors' opinion, the most important function of alpha-amylase in the bread baking process.

Summary

A method for the use of the amylograph for flour malt control is presented. The relation between this method and the maltose method was found to be generally poor. The same was found to be true of the relation between the amylograph method and the gassing power method.

The amylograph method was shown to be more sensitive to changes in malt activity and less sensitive to milling damage than either of the other methods.

The amylograph method is better suited to measure directly the primary effects of malt during the baking process than the maltose method and the gassing power method.

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COMMUNICATION TO THE EDITOR

A Corrected Unit of Alpha-Amylase Activity

SIR:

Shortly after the publication of our paper, "Modification of the Kneen and Sandstedt Methods for the Determination of Alpha- and Beta-Amylases in Barley Malt," *Cereal Chemistry* **21**: 533–539 (1944), we realized that an error had been included in the method of calculation of alpha-amylase activity.

In contrast to the unit of alpha-amylase activity originally proposed by Sandstedt, Kneen, and Blish (1939), the activity unit proposed in our work was based on an experimentally determined relationship between the dextrinizing and saccharifying properties of alpha-amylase. This relationship is given in Figure 1, page 536, of the above paper. The function, found to be linear over the range studied, did not pass through the origin; the method of calculation was based on the assumption that it did.

All attempts to redetermine the function resulted in curves of the same general type as that illustrated. The error over the entire range of the curve amounts to 15 maltose equivalents (4° Lintner), which is well within the limits of accuracy of the methods employed for the determination of saccharifying activity, but makes the values unsuitable for use as a measure of alpha-amylase activity.

The use of the relationship illustrated in Figure 1 and the values presented in Table I to correct the diastatic activity value for alpha-amylase saccharification, thereby obtaining values for beta-amylase, will not result in errors greater than those of the diastatic power method.

Therefore, we recommend the abandonment of the unit of alpha-amylase we originally proposed. We recommend further that a unit similar to that of Sandstedt, Kneen, and Blish (1939) be adopted, to wit: The alpha-amylase units are the number of grams of soluble starch which, under the influence of an excess of beta-amylase, are dextrinized by one gram of malt in one hour at 20°C, this unit to be called "20° dextrinizing unit."

Inasmuch as the only essential difference between the Nebraska methods and our modification is the temperature of extraction of the malt and of the dextrinization, it seemed desirable to establish a conversion factor between 20° and 30°C dextrinizing times. It was found that the conversion factor obtained with 30 barley malts [1] was not constant, often varying from the mean by 20%. Therefore, at present, we cannot recommend the use of a conversion factor between values obtained by the 20° and 30°C methods.

The calculations of the activities are the same as reported in our paper, except that alpha-amylase is calculated as indicated below:

$$\text{D.P. (maltose equivalent, dry basis)} = \frac{(\text{blank-titration}) \times 144 \times 100}{(100 - \%\ \text{moisture})}$$

$$\text{Alpha-amylase activity (20° dextrinizing unit dry basis)} = \frac{0.4 \times 60 \times 100}{\text{malt equiv. (g)} \times \text{dext. time} \times (100 - \%\ \text{moisture})}$$

Beta-amylase:

Correction for alpha-amylase saccharification (A)

$$A = \frac{K \times 100}{(100 - \%\ \text{moisture}) \times \text{ml diastatic extract used in dextrinization}}$$

and beta-amylase activity (20°C) maltose equivalents, dry = D.P. − A.

W. J. Olson, Ruth Evans, and A. D. Dickson
U. S. Department of Agriculture
Agricultural Experiment Station
Madison, Wisconsin

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Sandstedt, R. M., Kneen, E., and Blish, M. J.
1939 A standardized Wohlgemuth procedure for alpha-amylase activity. Cereal Chem. 16: 712–723.

[1] The results of this study will be submitted for publication shortly.

BOOK REVIEWS

Air Conditioning in the Baking Industry. By J. R. Seville. 152 pp. MacLaren & Sons, Ltd., London. 1946. Price 25 shillings in Great Britain.

This book deals with air conditioning from the baker's point of view. It attempts to give the "why" as well as the "how" as regards temperature and humidity controls. It contains 38 figures which are largely excellent photographic reproductions of equipment in use for recording and controlling the factors under discussion and including also bakery machinery used in the various stages of dough processing.

Section one of the book is introductory and general. It contains the following chapters: (1) Introduction, (2) The Basic Principles of Air Conditioning, (3) The Measurement of Humidity, (4) Obtaining the Required Humidity.

Section two of the book deals with Air Conditioning Applied to Breadmaking Processes. Each process or department is considered in a separate chapter and in the following order: (5) Flour Storage, (6) Doughroom, (7) First Proof, (8) Final Proof, (9) Oven, (10) Cooler, (11) Bread Storage. A unique part of this section of the book is the division of each chapter into two parts. The first part includes a considerable discussion of changes which occur during this particular part of the breadmaking process and the importance of temperature and humidity control in obtaining best results. The second part of each chapter describes suitable air conditioning methods and equipment for use in this operation. Chapter 12 contains notes on the Care of Air Conditioning Equipment. A glossary of terms most frequently used in air conditioning engineering is included. Tables are also given for temperature conversion, for finding relative humidities from wet and dry bulb readings, for showing relationships between velocity pressure and air velocity, and for pressure effects upon temperature, heat value, and volume of steam.

The author shows considerable familiarity with American methods and equipment. Much equipment from manufacturers in America and Britain is in use in both countries so the overseas authorship does not limit its usefulness on this side of the Atlantic. Descriptions of processes and equipment are clearly given in relatively simple language. Many cereal chemists, as well as bakers, might find this book to be a source of useful information. There may not be agreement with the author's opinions on some of the changes occurring in breadmaking. His views are rather positively stated, but this gives the presentation a certain simplicity.

OSCAR SKOVHOLT
Quality Bakers of America Cooperative, Inc.,
New York, N. Y.

Handbook of Analytical Methods for Soybeans and Soybean Products. National Soybean Processors Association, 3818 Board of Trade Bldg., Chicago, Illinois. 1946. 40 pp. Price $1.00.

This handbook is an example of a commendable practice that is gaining favor with trade associations. To facilitate the handling of raw and processed materials and to protect buyers and sellers in contracts for future delivery, such trade associations have set up specifications and trading rules, and have chosen official referees. To complete such a program it is necessary to stipulate precise directions for carrying out necessary analytical procedures; this handbook represents such a compilation for use by soybean processors.

The methods given are conventional procedures that fall within the limitations established by such organizations as the Association of Official Agricultural Chemists and the American Oil Chemists Society, but for the most part are given more specifically than is the case with methods published by the latter organizations. The A.O.A.C. and A.O.C.S. must necessarily write their official procedures in such a manner that some latitude is allowed for the judgment of the analyst in order that the general procedures may be satisfactory for use with a variety of products.

The one exception noted to the avowed editorial policy of eliminating alternative steps or procedures is in the determination of crude protein. Here the Kjeldahl-Gunning-Arnold method as given by the A.O.A.C. has been specified. It appears to the reviewer that for trading purposes the National Soybean Processors Association might well adopt a version of this method which would meet A.O.A.C.

requirements in all respects but which would be more specific in regard to such items as size of sample, choice of catalyst, and quantities of reagents to be used.

The Technical Committee of the N.S.P.A., which was responsible for the preparation of this handbook, has included several methods for the analysis of soy flour and lecithin which are not to be found in "official" compilations of methods. These procedures have proved their value in various plant laboratories, and should be welcomed particularly by those who have occasion to analyze soy products infrequently.

The style employed for presentation of methods is clear, and the booklet appears to be remarkably free from typographical errors.

F. C. HILDEBRAND
General Mills Inc.
Minneapolis, Minnesota

Concise Chemical and Technical Dictionary. Edited by H. Bennet. xxxix + 1055 pp. Chemical Publishing Co., Inc., Brooklyn, New York. 1947. Price $10.00.

This book aims at providing concise definitions of about 50,000 terms employed in every technical and scientific field. It includes thousands of trade-name or proprietary products together with a description of their chemical and physical properties and chemical composition. Several tables are included as well as an addenda listing the latest trade names and definitions of additional technical terms.

The system of nomenclature used for organic compounds is based on that recommended by the International Union of Chemistry.

The book is well printed but it is felt that greater consistency could have been observed in the cross references. Further, after using the dictionary for several weeks it is felt that it lacks many useful technical and scientific terms, especially with regard to cereal chemistry and the milling and baking industries.

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Why ENRICH Cereal foods?

OTHER WINTHROP NUTRIENTS

- **VITAMIN B_1** (Thiamine Hydrochloride)
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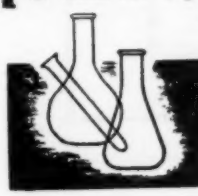

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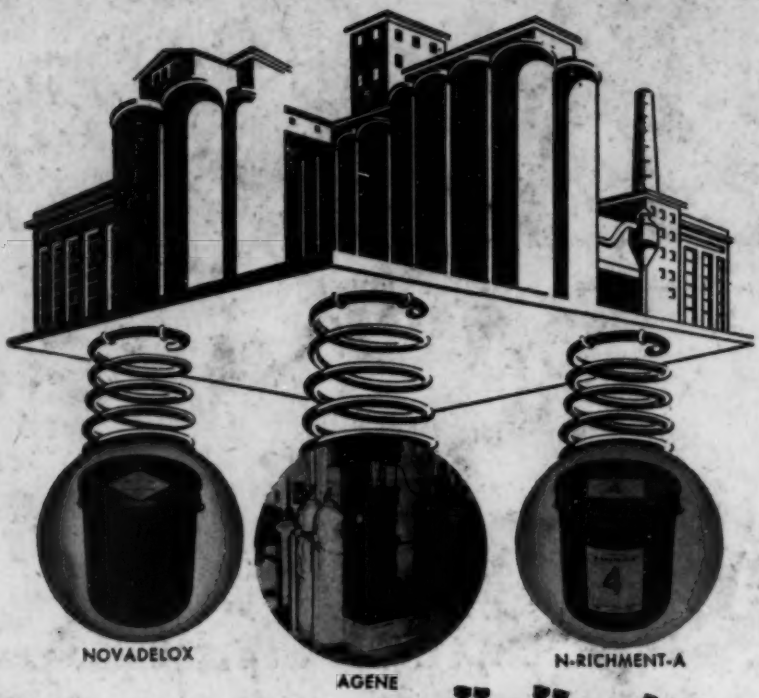

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